

A tale of two companies

The British government has made a muddle of its decision to prevent one electronics company from taking over another. It should pay more attention to what happens in the United States.

By an historical accident dating from the nineteenth century, Britain and the United States have in common not merely some usages of the same language, but also the existence of two public companies (corporations), one British and one American, that are called the General Electric Company. Both companies owe their origins to the demand, at the turn of this century, for machines for the generation, distribution and use of electric power. With the passage of time, both have become hugely successful and enormously diversified. Each, for example, has an interest in the manufacture of military aircraft, no doubt because the traditional manufacture of steam turbines for electricity generating sets provided a natural opening into the manufacture of jet engines. Inevitably, each has also an interest in consumer electronics.

The parallel between the British and the US companies has recently been even more striking; both have been seeking to grow by the acquisition of others. This is the spirit in which, earlier this year, GE of the United States merged with RCA Inc., itself a diversified conglomerate. It is also the spirit in which the British General Electric Company (GEC Ltd) had sought to merge with the smaller but still very substantial company called Plessey. The difference, between the United States and Britain, is that the British government has now used its anti-monopoly regulations to deny GEC the opportunity to pursue its commercial bid for Plessey.

Why should the British and US governments react so differently? Both governments are rightly concerned that companies should not become monopolists in their markets, charging their customers prices unrestrained by competition and serving the while as brakes on technical innovation. In the United States, the proposed acquisition of RCA by GE was formally studied by the US Department of Justice, which decided that the huge scale of the proposed merger was not in itself objectionable and that the fields in which the interests of GE and RCA most nearly coincide (defence and consumer electronics) are so competitively occupied by others that the merged company is unlikely to be a monopolist. In Britain, the proposed merger between GEC and Plessey was referred for consideration to the quasi-judicial Monopolies and Mergers Commission, which has the duty to consider the monopolistic risks of circumstances referred to it by the government, and whose conclusions may either be accepted (and then enforced) or, alternatively, may be rejected. On this occasion, the monopolies commission came out against the merger. The Secretary of State for Trade and Industry last week accepted this conclusion, the zeal with which his civil servants argued in the opposite direction when presenting their department's evidence to the commission notwithstanding.

Lessons

It will not be long before those concerned with this decision will be wishing they had behaved differently. The report of the monopolies commission, and the British government's ready acceptance of it, is a sign that people in Britain who should know better have not yet learned the lessons of the past few decades of industrial decline. The idea that GEC and Plessey should be merged is, ironically, itself a direct consequence of the policies

of past and present governments towards telecommunications. Once upon a time, nearly two decades ago, the then nationalized telecommunications authority set about the development of digital electronic switching systems (called trendily "System X") by inviting manufacturers to belong to a joint "research committee" constituted so as to persuade them to help pay for the high cost of developing such a system by promising them a share of the business that would eventually result, both domestically and from export sales. In the event, that enterprise has been a cruel disappointment. Of the original group of manufacturers, only GEC and Plessey survive. System X compares reasonably well with other modern equipment, but has been much delayed and is unlikely to command the huge export markets once talked of.

Worse still, for the companies concerned, their once-cosy relationship with the monopoly customer for telecommunications equipment has been undermined by the decision, fully implemented only two years ago, that British Telecom should become a commercial company. Not surprisingly, this monopoly customer now says it does not wish to have to depend on the old ring of suppliers. Now it plans to order some switching gear from other than British companies.

Rationalization

Accordingly, it is generally agreed, even by the monopolies commission, that GEC and Plessey must do something to rationalize their interests in System X. Indeed, the proposal that one should buy the other appears to have arisen directly out of the abortive talks between them over the past few years. What the commission now suggests is either that one should sell its switching business to the other or that they should jointly set up a separate company to handle their combined interest in digital telecommunications. The commission's report (Cmnd 9867, HMSO £9.80) unfortunately says nothing of the obvious snags. Is either of the two companies likely permanently to opt out of a field of technical development of such manifest importance, and would it be in the British national interest if that could be arranged? Even the suggestion that these two independent companies should be forced into a joint venture under the banner of a third company has little to be said for it, given the well-known instability of shotgun marriages and the near-certainty that both parties to such an enterprise intended merely to market a particular digital switching system would promptly (and sensibly) lose interest once a more attractive venture came along. If telecommunications were the only issue, the case for encouraging one company to buy the other would be irresistible.

So why should the argument have gone the other way? What seems most of all to have weighed with the monopolies commission, and eventually with the government, is the protest by the British Ministry of Defence that a merger of GEC and Plessey would give the combined company a virtual monopoly of the capacity to supply particular kinds of equipment, with the result that the ministry would not be able fully to implement its present policy of buying as much as possible of the defence equipment it needs only after companies working in the field have put in competitive tenders. But this argument is humbug.



The ministry is right to wish to buy its defence equipment as cheaply as possible, but is wrong to advance, for the purposes of a monopoly investigation, the argument that there have to be two potential British suppliers of everything it may choose to buy. The monopolies commission might just as sensibly have invited the defence ministry to follow British Telecom in listing overseas suppliers among those competing for its business.

This is the nub of the difficulty the British government has now created for itself. Although GEC is among the largest of British companies, it is tiny by the yardsticks of its direct competitors elsewhere, with annual sales roughly one sixth of the new combination of GE and RCA in the United States. Yet what the past few years have shown, specifically in the development of digital switching systems but generally, in electronics as a whole, is that the costs of launching new projects are invariably huge. Moreover, speed is of the essence; there are no prizes for being second. The point has probably now been reached at which only huge companies with long pockets can hope successfully to launch new projects that are technically interesting and likely to change the world in important and profitable ways. The sad history of System X shows the penalties of spending too little too late.

The British government, which for most of its spell in office has stuck to the principle that governments should not second-guess entrepreneurs, has on this occasion allowed itself to make industrial policy by default, by tamely accepting what the majority of the monopolies commission had to say (there is a dissenting minority report). The wisdom of this easy course will never be directly tested, for there may not now be a chance to tell what a slightly larger company than GEC might be able to accomplish by way of technical innovation. But there can be no doubt of its inequity; under British as distinct from US law, there is no way in which such decisions, however unfair they may be to shareholders of companies such as GEC, can be tested in the courts.

The moral in this sad tale is that all governments should take this opportunity to look again at their arrangements for regulating monopolies. In Britain, it is plainly an expensive luxury that companies are prevented from doing what makes economic sense for the sake of giving the defence procurement people the choice of several competitors for defence contracts; if that is what they believe they need, they should be prepared to throw the bidding open to all comers. In most European countries, there is an urgent need to ask how national legislation on monopolies can be squared with the general doctrine that trade flows freely across what used to be national frontiers. And to the extent that the United States now feels as much threatened by Japan as by the danger that its own companies might rig the domestic and overseas markets to their own advantage, there is a strong case for asking that the full rigours of the anti-trust legislation should be softened in the interests of the ultimate beneficiaries — those who buy the products of the new technology. □

Nuclear regulation

The International Atomic Energy Agency has handled Chernobyl deftly. What next?

THIS is bound to be more than an unusually formative year for the International Atomic Energy Agency (IAEA) at Vienna; the next few weeks could tell just what the agency's future holds. This is the season at which the agency's board of governors holds its annual meeting, in recent years a more or less routine affair concerned with the difficulty of recruiting enough skilled inspectors to operate the safeguards system and with the constant anxiety about the budget (which amounted to \$87 million last year, modest for a UN agency). The difference this year is Chernobyl and its consequences, both immediate and for the future development of nuclear energy.

How has the agency, by instinct (and formal constitution) a sponsor of nuclear energy, handled the past few weeks? First, it has done well to ensure a reasonably regular and frequent flow of information about the damaged reactor at Chernobyl. After ten tense days from 26 April, pressure from the agency seems to have been instrumental to that end, while its constitution as an international agency appears to have made it acceptable to the Soviet authorities and other governments in Eastern Europe as a repository of information. More recently, the agency has done a valuable job in persuading member states to give some thought to the steps that need taking to keep each other alert to the risks of cross-border pollution by radioactive materials; in due course, these arrangements may have the force of a convention. Later this month, the agency hopes to go one important step further with the consultation that has been arranged at which the Soviet side says it will tell all about the accident at Chernobyl.

Sponsor of nuclear power though the agency may be, it plainly recognizes that its sponsorship will lead nowhere if ordinary people in its members states are left with the dark suspicion that each nuclear power station is potentially as much a source of catastrophe as was Chernobyl. That is the only sensible view to take. The agency's problem, in the years that lie ahead, will be to blend realism and optimism in a convincing way. That will be a formidable task. □

Deeper pork barrels

Is the academic community in the United States too exercised by Congress's wayward ways?

FOR the past few years, the US Congress seems to have become an embarrassment to many in the academic community who are resentful of the notion that research funds should be distributed by congressional whim rather than by the more familiar techniques of judicious peer review and solemn committee discussion. Last year, Cornell University turned down the offer of a research grant it had not sought and in the process won the approval of those who believe that the award of money for research is a process far too serious to be left to the hunches of people whose only achievement is merely to have run successfully for public office. Most other recipients of these funds, which amount in total to rather more than \$10 million in any year, but probably less than \$50 million, have kept the money but have not advertised the fact. This year, in the rush to finish off the new tax bill and the budget for the financial year beginning in just six weeks, it is inevitable that many proposals for the support of specified research projects will finish up as amendments which are added almost surreptitiously to legislation making its way through the Congress for other purposes.

The US research community will do itself very little good if it takes too prim a view of these developments. Naturally, it would indeed be disastrous for the pattern of research in the United States if any but a small proportion of the funds available for research were to escape the standard system of scrutiny, or if the only means open to researchers for recruiting support for their work required, in the first instance, close personal acquaintance with an elected member of the Congress. But the sprinkling of research grants so far awarded from the pork barrel into which Congress from time to time dips hardly amounts to a substantial threat to the system as it stands. To the extent that most of the grants awarded in this informal way stem from the personal concern (however self-interested) of a congressman for a local academic institution, their frequency may even be regarded as a welcome sign that legislators are not as indifferent to the needs of the research community as they are sometimes said to be. Provided that the frequency of these grants does not get out of hand (in which case the spending agencies would no doubt decline to accept the instructions foisted upon them), there is a case for regarding them as a welcome way of bridging gaps in the orthodox system. Is not plurality still a benefit? □

NASA

US space station heads for deep freeze

Washington

BUDGET pressures on the National Aeronautics and Space Administration (NASA), together with management changes recommended by the Rogers commission and astronauts' heightened concern about safety, are forcing a major rethink of the agency's plan to build a habitable space station by 1994. NASA Administrator James Fletcher has ordered a thorough re-evaluation of current plans to take account of questions raised both by astronauts and by members of Congress sceptical of the feasibility of the dual-keel design announced in April.

Worries about safety and feasibility have become enmeshed in a political row over NASA's plan to transfer development of the station's crew modules from Johnson Space Center in Texas to Marshall Space Flight Center in Alabama; much of the criticism of the present design has come from Johnson. The subcommittee in the House of Representatives that oversees NASA has directed the agency to delay implementation of Fletcher's proposals, and congressional representatives from Texas queued up in Congress last week to lambast the new management plan and to demand greater consultation.

The astronauts' primary concerns are that the current design includes no provision for an escape system and that construction will require too much extra-vehicular activity by astronauts. A simpler one-keel structure could, it is argued, be

built at less risk. Astronaut Gordon Fullerton, in a briefing paper for NASA officials, also said that morale on the space station programme is poor and that there is concern that NASA is misrepresenting what it can do to Congress. Fullerton called for development of a new heavy lift vehicle in conjunction with the Department of Defense that would be used to lift the core components on the space station. A radical reappraisal of the space station has also been called for by Senator Spark Matsunaga (Democrat, Hawaii), who favours separate government and commercial stations with different design objectives.

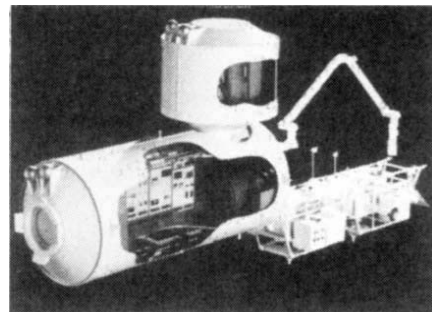
NASA officials say that although reviews of alternative construction plans are in progress, the basic dual-keel design formally proposed to Congress last April still stands. Provision of an escape module or a "safe haven", and a revised construction plan that would allow for earlier use of the space station, are among the options being studied. But Fletcher told Congress last week that the current design could be built only if a fourth shuttle orbiter to replace the Challenger is built. The administration should soon announce how a fourth shuttle would be paid for.

The extent of European participation in the space station is also still far from clear. The European Space Agency (ESA) is adamant that the permanently attached pressurized module for which preliminary design studies were agreed on 1 August

Japanese space plans go ahead

Tokyo

DESPITE uncertainties over the future of the US space station, Japan's Space Activities Commission has just released a report recommending continued "positive" participation. Over the past two fiscal years, ¥5,500 million (\$36 million) has been allotted to the design of an experimental space module (above) to be attached to the space station, and the report recommends that construction of its main frame should



begin in 1989 with a view to launch in 1994.

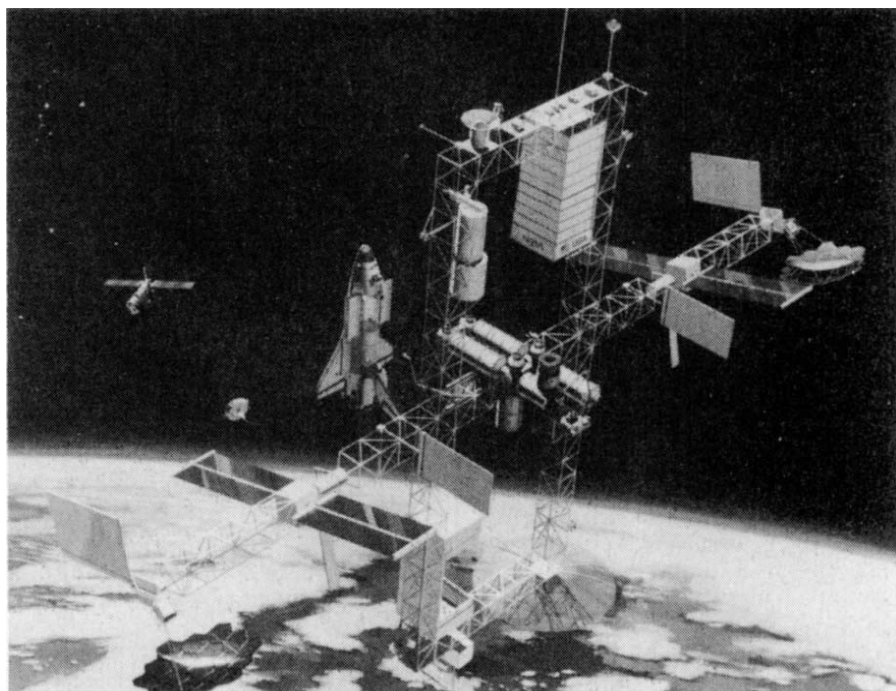
The module will consist of a pressurized cabin ten metres long for use in experiments on new materials under gravity-free conditions, an exposed service platform equipped with a manipulator and a storage cabin. One or two astronauts will man the station and the report urges the government to begin training in earnest. The estimated cost of the module is ¥350,000 million (\$2,300 million). David Swinbanks

will be used mainly for materials research, where profitable spin-off technologies are most likely to emerge. But a congressional committee that approves NASA's budget recently recommended that \$150 million of the \$410 million sought by the agency for space station development next year should be reserved until certain conditions had been met, among them that ESA's pressurized module will be restricted to life science research.

The 1 August agreement indicates that ESA will also conduct preliminary design work on a man-tended free-flying pressurized module that could dock on the space station, as well as a polar orbiting platform. In return, NASA has agreed that it will study the man-tended free-flyer module jointly with ESA. This is essentially a compromise to allow ESA to continue work on the free flyer, which it has favoured all along as its primary contribution but about which NASA is unenthusiastic. The interim agreement covers only preliminary design.

All concerned acknowledge that the NASA/ESA agreement has merely allowed work to continue and has not resolved fundamental differences of approach between the two sides.

Tim Beardsley



European Commission

Framework to be scaled down

AMBITIOUS plans for a major expansion of European research and development are cut back by 30 per cent in the latest proposals from the European Commission. The European "framework programme" was to have cost in excess of

Europe will generate support. There is plenty of competition, however, from independent projects such as Eureka aimed at stimulating Europe's high-technology industries.

The new price-tag for the framework programme is 7,735 million ECU. The adjustments have been made to balance the differing national interests expressed at the last research council meeting: West Germany is reluctant to go into joint telecommunications research, where its principal company, Siemens, feels it has more to lose than to gain, while the British insist on continuous programme assessment by outside experts.

Newer activities, such as information technology for the disabled, where the Commission has less experience, are also being cut back. A programme for research on "intelligent transport", involving advanced computers and communications in road vehicles has been cut because of strong overlap with a Eureka programme. New technologies for old industries are also out of favour. But energy research sees a slight increase in response to the

Chernobyl disaster. The whole of the Commission's nuclear fission research programme, much of it at the Commission's own Ispra laboratory in northern Italy, is now concerned with nuclear safety.

Will member states support the new proposals for the programme? Britain, whose technology minister Mr Geoffrey Pattie will be influential as he chairs the council of research ministers until the end of this year, believes there is room for an increase in total research spending, but not such a substantial one as the Commission is requesting, even in its revised budget. Commission officials certainly believe the larger member states, which pay most of the Commission's bills, are going to be reluctant, but will argue that everybody's long-term interests will be served by improving European cooperation in this area. Britain, however, with its research community in revolt at low levels of support at home, will be nervous about the impact of increased international spending on its national research budgets, a matter which is not entirely in Pattie's control. But with the Commission's final proposals now in the hands of national governments, real negotiation can begin.

Robert Walgate



10,000 million ECU (over £15,000 million). But European research ministers, meeting in June, protested when they saw the budget was more than double the cost of research at present being sponsored by the Commission.

Commission officials had hoped for better progress on the Common Agricultural Policy (CAP), which absorbs more than two-thirds of total Commission spending in farmers' subsidies. If CAP had been trimmed, that could have left room for a larger research programme, now representing only 2 per cent of national research and development budgets. Instead, CAP has grown, the Commission budget has been pushed to its legal ceiling (1.4 per cent of member states' value added tax receipts), and smaller programmes have little chance of gaining extra funds.

Commission research, mostly performed in national, university or industrial laboratories, is strongest in energy (including nuclear fission safety research and fusion), the environment, information and communications technologies, biotechnology (particularly in relation to agricultural change and development), new materials, optical computing and technologies thought likely to revive old industries. Its objective is to unite European research groups on long-term projects and reduce duplication in national programmes. The framework programme is designed to set priorities for the next five years. Two years ago, when planning began, major expansion seemed both politically and financially possible. Now that CAP has eaten the cupboard bare, the Commission's research directorate must hope that increasing political interest in technological cooperation in

Israeli science

Medical schools seek US students

Rehovot

US MEDICAL students at Tel Aviv University pay tuition fees of \$15,000 a year, a lot more than their Israeli counterparts, who pay only \$900 (a remarkably low sum decided on by the government). Yet there are far more US applicants for the programme in Tel Aviv than can be accepted, says Yehuda Bar-Shaul, rector of the university. That, he says, is because "the special English-language course at Tel Aviv is not like those of the dubious 'off-shore universities' which many American students attend". The Tel Aviv institution has the status of a New York state medical school, and participants in the course who come from New York have more than 40 per cent of their tuition paid for by the state. Moreover, after graduation, the students do their residency in New York hospitals, "and the very best ones at that", the rector adds with pride.

Each year, Tel Aviv University's Sackler Medical School takes in some 60 students from the United States and about 80 from Israel. The US intake causes resentment among Israelis whose applications are rejected and who must therefore go to Italy or Rumania in order to study medicine, but, Ben-Shaul says, there is no justification for such resentment. "Even if this special programme were abolished", he declares, "we would not accept more local students because the 300 new doctors

who graduate each year from the country's four medical schools are more than sufficient to meet Israel's needs."

The example set by Tel Aviv University over the past six years is to be copied by two other major institutions. A few weeks ago, the Haifa Technion decided in principle to begin a similar scheme at its own medical school. But Technion officials speak of fulfilling a Zionist mission, since most of those who would come to study in Haifa are likely to be Jews (as is the case in Tel Aviv). Officials readily admit that they also see the course as a potential source of income for the financially troubled Technion, which will be able to accept overseas students and their tuition fees without investing money in new facilities.

A similar scheme is on the agenda at the newly established Korat School of Veterinary Medicine of the Hebrew University. Its director, Professor Kalman Perk, expects an annual intake of 30 students, half Israeli and half foreign. But the programme will begin only in two years' time, after the opening of a veterinary hospital near the campus and after the first veterinary school class has graduated.

Unlike Tel Aviv University and Technion, overseas participants in the Hebrew University scheme will be expected, after the first year, to attend regular Hebrew-language classes.

Nechemia Meyers

Strategic Defense Initiative

Japan ready to participate

Tokyo

BOUYED by its landslide victory in the last Japanese election, the Nakasone administration is galloping to join the US Strategic Defense Initiative (SDI). The last barrier to participation, an all-party resolution banning the militarization of space, has been brushed aside and Japan is expected to decide to join the SDI research programme by the beginning of September. But there is less enthusiasm among those who will actually carry out the research.

In March last year, Mr Caspar Weinberger, US Secretary of Defense, formally invited Japan as well as other allies to participate in SDI. Since then, three Japanese delegations have been dispatched to the United States, the last of which, after its visit in April, strongly recommended participation on the grounds that SDI research would greatly benefit Japanese industry (see *Nature* 321, 5; 1986).

Since the July general election, two cabinet-level meetings on SDI have been held and several key decisions have been made. First, all patent rights to technology developed through SDI research will pass to the United States, although Japan will try to work out an agreement to ensure the return of technology to Japan. Without such a provision, the prime incentive for Japan's private industry to take part would be lost, as the main benefits for Japan are seen to lie in commercial spin-offs from SDI.

Second, although most research will be carried out by private enterprise, government agencies will be allowed to join the research effort, and secrecy will be maintained under the existing Secrets Protection Law. Finally, the six cabinet ministers, at their meeting last week, concluded that participation in the research phase of SDI would not violate a 1969 all-party Diet resolution banning military use of space. Defence-related issues usually provoke heated debate in Japan, and last week's cabinet decision would normally raise a storm. But opposition parties have remained remarkably silent on SDI following their crushing election defeat.

Dissent within Prime Minister Yasuhiro Nakasone's own Liberal Democratic Party (LDP) has also failed to materialize. Former Foreign Minister Shintaro Abe, a contender for the LDP leadership, is known to be cool towards SDI, but in his new post as chairman of the LDP executive council he no longer has a direct say in SDI and his successor at the Foreign Ministry, Tadashi Kuranari, is a close political ally of Nakasone. Nakasone himself has made no secret of his enthusiasm for SDI since he first discussed the issue with President Reagan last year, and a decision to go ahead would no doubt

strengthen the friendship between the US president and the Japanese prime minister.

Private industry's enthusiasm for SDI is more guarded. There are fears that it could merely end up being exploited, without enjoying the "fruits" of the research, and the decision to transfer patent rights to the United States will do little to allay such concern.

Although government research agencies

will also be able to participate in the SDI programme, there may be a lack of scientists willing to carry out the research. Nearly 5,000 Japanese scientists have signed petitions against SDI, including more than 1,600 at the national science research laboratories in Tsukuba, the very laboratories at which government SDI research is likely to be carried out. The scientists' chief concern seems to be that their research would be restricted as "military secrets", but they also fear an arms race in space and the increased dangers of nuclear war.

David Swinbanks

Environmental pollution

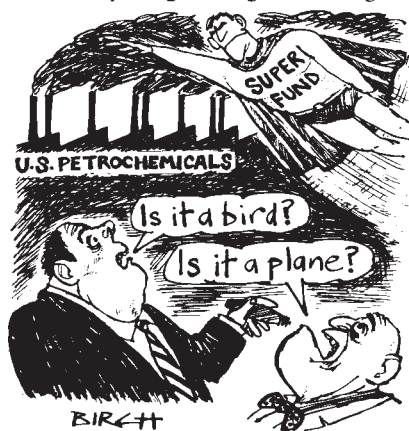
Pushing for a new clean-up law

Washington

AFTER languishing in legislative limbo, the US Comprehensive Environmental Response, Compensation and Liability Act, better known as the Superfund, is on the road to reauthorization. A conference committee last month completed the task of reconciling differences in new authorizing legislation already passed by the House of Representatives and the Senate.

Congress established Superfund in 1980 to clean up hazardous waste sites created before the passage of legislation regulat-

ing the dumping of such wastes. Superfund's first five-year authorizing legislation expired last October. The Environmental Protection Agency (EPA), responsible for Superfund clean-ups, has kept the programme going with residual funds and stop-gap appropriations from Congress. In the final version of the reauthorizing legislation, Superfund is authorized to receive \$9,000 million over the next five years, compared with \$1,600 million called for in the 1980 legislation.



One major obstacle to the implementation of Superfund is the issue of who will foot the bill for the clean-ups. The primary reason Congress failed to reauthorize Superfund when it expired last fall was an inability to come to terms with this issue. The Congress is in the process of working out a complete overhaul of the United States' tax code. So far, the issue of new

taxes to fund Superfund has not been taken up, and the issue is a divisive one.

The petrochemical industry has claimed that paying the entire cost for clean-ups could be devastating for the industry's international competitiveness. One alternative is a value-added tax.

Under the new statutes, a timetable will be established for clean-ups to prod EPA to move more aggressively than it has in the past. Over the next four years, EPA will review some 23,000 potential Superfund sites around the country, with some 2,000 sites likely to be added to the 888 at present on EPA's National Priority List. Federal clean-up standards, absent from the original legislation, appear now, along with a provision that allows state standards to apply if they are more stringent.

Partly in response to the chemical plant disaster in Bhopal, India in 1984 (see *Nature* 312, 581; 1984), companies either using or creating toxic chemicals in manufacturing processes must inform local jurisdictions of their activities. This community "right-to-know" database could become a boon for epidemiologists if it is established as planned, providing information about environmental hazards. The new rules also require the Department of Health and Human Services to compile a toxicology profile on the commonest chemicals at Superfund clean-up sites.

Some critics of the new bill are concerned that issues of corporate liability have still not been adequately dealt with. Others say it does not go far enough to solving the problems caused by hazardous waste sites. A report by the Office of Technology Assessment (OTA) suggested that it would cost closer to \$100,000 million to clean up all the dump sites that could be eligible for Superfund action. OTA's Joel Hirschhorn says that for the first time funds will be available for developing new clean-up technologies, but otherwise sees no major improvement in the Superfund law. "It's one thing to throw money at a problem", says Hirschhorn, "and another to solve the problem."

Joseph Palca

Computer models

Cooperation on new molecules

Washington

COMPUTER models are becoming increasingly popular for designing new molecules. But all such efforts face a fundamental problem: what parameters and potential-energy equations should be used in estimating the final energy state and geometry of a novel molecule? A recently formed consortium has set itself the ambitious task of supplying those parameters and equations. A successful outcome will mean model-builders will sleep easier, confident that their new creations will behave in nature the way they do on a computer screen.

The new three-year project is headed by Biosym Technologies, Inc., a company based in San Diego specializing in software for computer-assisted molecular design (CAMD). Each year, consortium members will contribute money and manpower: \$30,000 in cash support and six months of personnel time from a doctoral-level chemist. In return, consortium members have a say in the direction the

project takes, as well as gaining first access to the new database and any software tools that are developed. Companies participating in the consortium are Abbot Laboratories, Cray Research, E.I. du Pont de Nemours, Merck Sharp and Dohme Research Laboratories, Monsanto, Rohm & Haas and the Upjohn Company.

For certain classes of molecules, such as polypeptides and nucleic acids, these energy functions, known as force-field functions, have been fairly well approximated. The parameters are derived not only from bond energies, but from non-bonded molecular interactions as well. Peter Kollman, professor of pharmaceutical chemistry at the University of California, San Francisco, is the author of one widely used set of these functions. But Kollman says that, when looking beyond well-characterized classes of molecules, the applicability of the force-field functions becomes uncertain.

As an example, the van der Waals forces between molecules in two classes of compounds may be similar, but the electrostatic charges may differ, forcing a model-builder to make assumptions about the best way to alter the force-field equations. The consortium hopes to develop a more generally applicable set of parameters and equations.

Biosym's chief scientific officer, Arnold Hagler, has also developed a well-known set of force-field equations. Hagler's plan is to use both empirical data gathered from existing literature and research done by consortium members, as well as quantum mechanics calculations, to develop a "second generation database of potential energy surfaces for organic, pharmacological and other biomolecules".

While most consortium members are also fierce competitors in the marketplace, Upjohn's director of computational chemistry, Gerry Maggiora, explains that it will be to everyone's advantage to have a good set of potential energy functions. Upjohn is heavily involved in CAMD, one focus of which is developing inhibitors of renin for use as antihypertensive agents. As the three-dimensional structure of renin has not yet been worked out, Upjohn and others have turned to related compounds such as aspartyl proteinases to study molecules that will bind to renin's active site.

John Wendoloski of du Pont says companies should probably have put more money and effort into joint developments of force-field functions in the past. Although there is nearly uniform agreement that Biosym has undertaken a potentially useful task, there are those who doubt that a general set of force-field func-

tions can be developed at present. Cyrus Levinthal of Columbia University, who is developing a special application computer with Brookhaven National Laboratory for computing interacting forces between molecules, says it is not clear whether sufficient empirical data exist to generate appropriate parameters. That sentiment is echoed by Kollman, as well as by John McAlister, director of research for Tripos Associates, another software company also working on the force-field problem. Even consortium members confess that hoping for a "second generation" database may be optimistic, but Abbot's Jonathan Greer says a better set of parameters than those currently available will be welcome.

Joseph Palca

Return of tamarins proves expensive

Tokyo

THE World Wildlife Fund has agreed to an unprecedented payment of about 10 million yen (\$65,000) to ensure that ten golden-headed lion tamarins illegally imported to Japan three years ago return home to Brazil, according to the Japanese Foreign Ministry. Despite the acute embarrassment the illegal import of these rare primates has brought to Japan, the Japanese government is making no move to introduce legislation to prevent a repetition of such incidents, and has failed to provide funds for the safe return of the animals.

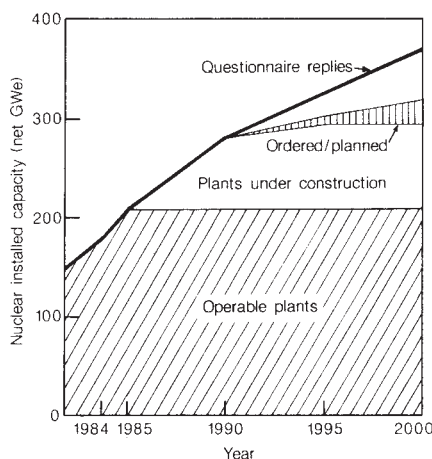
The weakness of Japan's laws has been quickly spotted by the *yakuza*, Japan's own mafia. Just a few weeks ago, they put a black tamarin on sale on the street outside a police station at Tokyo's Shinjuku station. Their sales point, capitalizing on media attention to the smuggling of endangered species, was that the tamarin had been brought in illegally from Brazil. No action was taken against them.

The lion tamarins, trade in which is banned under the Washington Convention on International Trade in Endangered Species, to which Japan is a signatory, were brought into Japan in 1983 with false import documents (see *Nature* 318, 200; 1985). The infringement was spotted by the World Wildlife Fund's TRAFFIC office. But, having got the monkeys past customs, the importers were free from prosecution as there is no domestic legislation to back up the terms of the convention.

Only constant lobbying by the World Wildlife Fund and international protests forced the government to take action. But it has no intention of paying the bill. According to the Foreign Ministry, the monkeys will be shipped to São Paulo Zoo in mid-September at a cost of 15 million yen, two-thirds of which will be paid by the World Wildlife Fund. The remaining one-third will come from the importer and keepers of the tamarins. David Swinbanks

Nuclear forecast

AMID Chernobyl gloom, the Nuclear Energy Agency of the Organisation for Economic Cooperation and Development (OECD) is still presenting an optimistic picture of the growth of nuclear power worldwide. In 1985, there was a 19.2 per cent increase in nuclear electricity production, the largest increase since 1977. Twenty-one per cent of electricity generated in the OECD countries comes from nuclear power stations, ahead of hydro-



thermal and geothermal (20 per cent) and oil and gas (17 per cent) but behind coal (42 per cent). Forecasts to the end of the century shown in the graph were prepared from questionnaires returned by member countries. The highest forecast includes power stations not yet at the planning stage, where worries over safety may have their effect. □

Seismic monitoring

Station goes with a bang

THE Soviet-US seismic monitoring expedition to Karkaralinsk in Kazakhstan is essentially a confidence-building exercise aimed at showing it is possible to detect infringements of any future nuclear test-ban treaty, according to Oleg Stolyarov, a laboratory head from the Schmidt Institute of Earth Physics of the Soviet Academy of Sciences.

The expedition has been a unique co-operative venture that has allowed a team of US scientists to set up a seismic monitoring station in the Soviet Union. The Soviet side will reciprocate later and help to set up a station in Nevada. But the US government has not been involved: the

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agreement is between the National Resources Defense Council, a private environmental group, and the Soviet Academy of Sciences (see *Nature* 321, 638; 1986).

Stolyarov, who has been acting as spokesman for the Soviet side, maintains that all the scientists on the expedition agree that seismic tremors can be monitored with sufficient accuracy to detect nuclear tests. The real purpose of the exercise, he maintains, is to convince the US authorities, who would prefer not to believe it.

Since the US participants do not have government backing, Stolyarov may have assumed that the government was hostile to the project. Other difficulties may have helped confirm this impression. As Co-Com regulations forbid the export of Western state-of-the-art technology to the Soviet bloc, the US equipment taken to Karkaralinsk was somewhat outmoded.

In the event, the Americans working at Karkaralinsk, a geological freak area where outcrops of bedrocks that are highly conductive to vibrations make it a natural seismic observation site, obtained excellent signals from nuclear blasts in Nevada.

Despite the political tensions generated by US reluctance to follow the Soviet example of declaring a nuclear moratorium, the two teams are said by Stolyarov to have "worked hard in a friendly way... brought together by a noble purpose".

Vera Rich

Ariane ride for Indian satellite

Bangalore

THE Ariane launch vehicle of the European Space Agency (ESA) will launch India's multi-purpose domestic satellite INSAT-1C in early 1988 according to an agreement signed between the Indian Space Department and Paris-based Arianespace. This agreement brings to an end uncertainty over the launching of INSAT-1C, originally planned for the space shuttle (see *Nature* 322, 198; 1986).

The INSAT spacecraft, described as the first civilian geostationary satellite to combine telecommunications, direct television broadcasting and meteorological observation tasks, are being built by the Ford Aerospace Communications Corporation (FACC) of Palo Alto, California, under a contract from the Indian Space Department.

With a launch weight of 1,180 kg, INSAT-1C is similar in configuration and performance to INSAT-1B, now in orbit. INSAT-1A, the first in the series, was launched in April 1982 but was switched off six months after its launch following a series of malfunctions. INSAT-1B went up in August 1983 and is running smoothly. All the spacecraft are designed for seven years active life in orbit. Successful launch of INSAT-1C will complete plans to have one operational satellite and one spare in orbit. INSAT-1D is expected to be launched in late 1988 or early 1989.

According to Professor U. R. Rao, secretary of the Indian Space Department, Arianespace offered the only competitive option after the shuttle failure had been followed by problems with the US conventional launcher, the Delta rocket. He said that the European launching system is as

good as the US system although it is a little costlier at US \$33 million. "But the French have gone out of their way to accommodate us", he said.

Frederic d'Allest, chairman of Arianespace, and Rao hailed the agreement as a

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milestone in the long-standing and steadily growing cooperation in the field of space between India and Europe in general and France in particular. They are confident of further expansion of Indo-French cooperation in other areas such as remote sensing, satellite-aided search and rescue for the benefit of both countries.

Meanwhile, India is busy designing the second-generation INSAT spacecraft which will be built and launched in India. A liquid oxygen/liquid hydrogen launcher is being developed. Radhakrishna Rao

Doing things the all-American way

Washington

A BILL that would limit European and Japanese participation in the US Strategic Defense Initiative (SDI) has passed the Senate, and will be acted on by the House of Representatives this week. In a rare Saturday session, the Senate adopted an amendment, sponsored by Senator John Glenn (Democrat, Ohio), to the defence authorization bill that would prohibit the SDI office from signing contracts with foreign companies unless the Secretary of Defense certified that the work could not be performed in the United States. The amendment does not cover work subcontracted to companies outside the United States.

In the House of Representatives, a similar amendment, sponsored by Representative Les AuCoin (Democrat, Oregon), would place a size limit of \$100,000 on SDI contracts with non-US companies. AuCoin's amendment does not permit ex-

ceptions. The House amendment is intended both to provide additional jobs for US workers and to prevent the Reagan administration from buying support for SDI from US allies with fat research contracts. It was impossible earlier this week to estimate the amendment's chances of success.

The Federation of American Scientists estimates that foreign SDI contracts at present amount to \$75 million. Although this represents only a small fraction of SDI's 1986 budget of \$3,050 million, some countries clearly hope for a much larger role in the project (see *Nature*, 322, 300; 1986).

The version of the defence authorisation bill passed by the Senate would limit spending on SDI to \$3,950 million, well below the administration's \$5,400 million budget request. That figure is likely to be trimmed even further in the House of Representatives' version. Joseph Palca

Chernobyl accident

Reactor design not perfect

ACADEMICIAN Vitalii Legasov, a deputy director of the Kurchatov Nuclear Energy Institute, last week said that the design of the RBMK reactor was partially to blame for the Chernobyl disaster. The official Soviet viewpoint, embodied in a Politburo statement last month, was that human "irresponsibility" and "lack of labour discipline" was the cause. Workers at the power station, the Politburo said, had carried out unauthorized experiments on the Number 4 reactor, when it was undergoing a routine shutdown.

Although he did not disagree with the accident scenario, Legasov added some important details. In an interview for Japanese television, he said that the workers had not only carried out the "experiment" without the appropriate experts being present, they had also committed the "unbelievable" error of "keeping the emergency cooling system out of operation".

The experiment, he said, was intended to ascertain how long a power supply (for operating the reactor's own housekeeping functions) could be maintained after switching to the standby diesel generators. Such an experiment, he maintained, should have been carried out only with the reactor completely shut down and steam generation stopped. This account is compatible with the speculations of Western experts (see *Nature* 322, 399; 1986), that attempts to draw energy from the reactor in these conditions, well below the designed power range for the RBMK, produced steam voids within the cooling system, which caused power surges that

Guatemala accused of political killings

Washington

THE American Association for the Advancement of Science (AAAS) has submitted to President Vinicio Cerezo of Guatemala a grim catalogue of more than 200 political killings, "disappearances" and arbitrary detentions of Guatemalan academics and students between 1980 and 1985. Most are believed to have been killed with the approval or the tolerance of the military governments that ruled Guatemala between 1978 and last January.

The reports prepared by AAAS's commission scientific freedom and responsibility, urges President Cerezo, a civilian, to investigate the cases and to allow families to seek judicial redress. Most of the cases describe scientific and medical professionals who were shot or abducted by unidentified men. Estimates of the total number of political killings in Guatemala over the past 15 years range up to 100,000.

After AAAS presented a similar report to the new civilian government of Argentina

in 1984 detailing disappearances of 55 Argentinian scientists, the Argentine government requested forensic science assistance from AAAS to investigate the cases. Cerezo has pledged to foster greater respect for human rights and to reform the national police and security forces, but those in the military responsible for unlawful killings cannot be brought to justice until an amnesty declared by the outgoing military government is repealed. It is not clear whether Cerezo will be able or willing to repeal the amnesty.

Plans for an independent human rights commission on Guatemala with international representation have so far come to nothing, but the government has indicated that it may be willing to appoint a special ombudsman to investigate allegations of human rights abuses. In the meantime, one judge has been appointed to hear 1,400 writs of habeas corpus submitted by Grupo de Apoya Mutuo, a local human rights group. Tim Beardsley

could not be controlled by the slow-moving control rods of this type of reactor.

Where Legasov differs from the official Soviet viewpoint is in maintaining that not only were the "experimenting" workers at fault, but also the design team, who should have foreseen these events.

For some time, Legasov has been suggesting that the explanation of the Chernobyl disaster would be a complex one. Interviewed last month for the Soviet journal *New Times*, he described it as a "scarcely probable thing . . . a combination of several events, each of them scarcely probable either". The idea that the designers should be able to predict accidents is not entirely new to the Chernobyl coverage; interviewed by the Mos-

cow weekly *Literaturnaya Gazeta* in June, Academician Boris Semenov, deputy chairman of the State Committee for the Use of Atomic Energy, said that the safety systems at Chernobyl were sufficient to prevent any escape of radioactive material in even the worst scenario foreseen by the designers — clearly implying that some possibilities had been overlooked.

By placing the responsibility on the Chernobyl workers, the Politburo statement implicitly exonerated the RBMK design, which is one of the two basic types used in the current Soviet nuclear power programme. During the past few weeks, official statements have made it clear that there are no plans to phase out or close down RBMK-powered stations in the immediate future, although there have been some exhortations to the scientists to produce a "new, safer" generation of nuclear reactors as soon as possible. A need to express government confidence in the RBMK may well lie behind the appointment to the new post of All-Union Minister of Nuclear Energy of Nikolai Lukonin, formerly director of the RBMK-power stations at Leningrad (1976–83) and Ignalina, near Vilnius (1983–86). Behind the scenes, however, the government and party leadership may be less happy with the design. One of the four top officials dismissed in July as a result of the official inquiry into the accident was Ivan Emelyanov, who some 20 years ago was head of the design team that produced the RBMK. Retraining programmes for the staff of nuclear power stations, including computer simulation of accidents and emergencies, are being introduced, and the siting of some projected power-station is now said to be under review.

Vera Rich

Japan's sunset export industry

Tokyo

THERE is nothing like an old solution for a new problem. For years, Japan's Ministry of International Trade and Industry (MITI) has been exhorting industry to export, export and export some more. Now the nation has a new problem: with the Japanese the longest-lived people in the world, there are soon going to be far too many old people. The MITI solution — export them!

Under the "Silver Columbia '92" plan, Japan's old-age pensioners will be able to set off to foreign lands where they can live in peace and comfort supported by an ever-rising yen. Life back home would be much harder, given the poor provision for pensions and the unwillingness of the government to take on any new financial commitments.

MITI foresees the establishment of

Japanese villages of around 1,000 people each, with construction beginning in 1990. Of course, a few problems remain. Next year, MITI and the Japan External Trade Organization will work out where the villages should be and ways of tackling language barriers and supplying Japanese food. Countries will be selected in the light of climate, law and order, medical services, housing and popular sentiment towards Japan.

Although the Japanese government would negotiate with the prospective host country, MITI wants the private sector to run the scheme. It is not yet clear how foreign governments will react. Will they see it as a welcome transfer of "intellectual property" or just another case of "dumping"? Could a trade develop? And would Japan be happy to import a few pensioners from elsewhere? David Swinbanks

Hubble space telescope

Every delay has a silver lining

Baltimore, Maryland

THAT the launch of the Hubble Space Telescope seems likely to be delayed until 1989 may yet turn out to be a blessing in disguise. Many project scientists believe that the three-year delay, caused by the Challenger space shuttle accident, will give time to make the telescope more



Riccardo Giacconi

effective — provided the National Aeronautics and Space Administration (NASA) can avoid making major cutbacks.

Samuel Keller, deputy associate administrator of NASA's Office of Space Science and Applications, says the agency hopes to avoid redundancies at the Space Telescope Science Institute (STSI) in Baltimore, but that a 10–20 per cent fall in staff numbers is likely, due to natural wastage. No new staff are being taken on. At Marshall Space Flight Center in Alabama, staff reductions have taken effect. As Keller points out, NASA has to comply with levels of funding and directives imposed by Congress.

The telescope's large size (43 ft) and weight (around 24,000 lb) mean that only the shuttle could put it into space. NASA recently announced that the shuttle will not fly before 1988, but Dr Riccardo Giacconi, director of STSI, believes it "still reasonable" to hope for launch during 1988. Giacconi thinks the telescope could be the third to the fifth payload in line once flights resume. The first will probably be military, and the second a tracking and data relay satellite to replace that lost with Challenger (one such satellite is now in operation for dual military and civilian use, but at least two are needed for safe control of the telescope).

Keeping the telescope waiting is not likely to cause problems. But the delay will necessarily mean large cost increases at Lockheed Missiles and Space Corporation in California, where about 150 people are testing the telescope. NASA has considered stopping work until close to launch, but it would still be necessary to keep an experienced engineering team together for pre-flight tests. Giacconi now thinks he has NASA's support for keeping the telescope accessible for testing until launch, but major reductions in the

Lockheed workforce are still likely.

There is clearly still much to do. The main controlling system, the space operations ground system, is installed in a basic form, but the final version will not be delivered by TRW Inc. until the end of the year because of development delays. In its present form, the system could not, for example, control the telescope so as to track moving targets such as planetary moons. Other systems, such as that which will calculate optimal timing of observations, are still in development. Some upgrading of hardware at STSI is also now thought to be necessary, but their cost, though counted in hundreds of thousands of dollars, is small compared with the software. All agree that it will be easier to complete installation and testing of complicated control systems with the instrument still on the ground.

Giacconi also wants to use the delayed deadline to allow institute astronomers to catch up on their own research and to foster a "more academic atmosphere". Although the official target for institute staff has always been that they would

Weight problems

IMPROVEMENTS to the shuttle recommended by the Rogers commission investigation of the Challenger accident will add weight and could impair the shuttle's ability to put the massive Hubble Space Telescope into a high enough orbit. Although the ideal height would be 360 nautical miles or more, even the unmodified shuttle is unlikely to manage 320 nautical miles.

At this height, atmospheric drag would gradually bring the telescope down: the plan was that a shuttle flight two years or so after launch would boost the telescope up to the desired 360 miles. (Some point out that if NASA by then has an operational orbiter transfer vehicle — at present still on the drawing board — the telescope may even be taken up to 400 nautical miles, where it would be stable indefinitely.) But if the modified shuttle is incapable of getting close to 320 nautical miles, there may be problems caused by orbital decay and atmospheric interference.

NASA officials Fred Wojtalik of Marshall and Frank Carr of Goddard Space Flight Center do not expect there will be problems, however. Keller, at NASA headquarters, points out that the weight of improvements to the shuttle has not yet been determined and thinks the rumoured figure of 12,000 lb may be pessimistic. The more modest weight increases he thinks likely would, he estimates, result in an orbit only 10–15 miles lower than that now planned.

Tim Beardsley

spend 50 per cent of their time on research, in February the figure was still less than 25 per cent. Proposals for guest astronomers will not now be taken until March 1987 to take account of the launch delay.

Last June, the telescope successfully completed its first major test: a thermal vacuum test which used a simulated solar source to investigate heating effects, and other calibrated light sources for basic checks of the major instruments. Although many minor problems were found, none require extensive redesign or rebuilding. The test did, however, indicate that the telescope takes longer than expected to reach thermal equilibrium, necessary to avoid structural distortion. Among solutions being considered are extra insulating material and more solar cells to make more use of heating elements. The thermal vacuum test also included the first end-to-end test of the telescope's science operations and data analysis system.

Commands were successfully sent from STSI via Goddard through a simulated tracking and data relay satellite to the instrument itself. Although STSI sees the test as a major success, it is only a beginning. Just three of the main instruments were tested, and because of the incomplete control system and software incompatibilities with Lockheed, many of the commands had to be translated manually.

STSI has in the past been criticized for failure to control its budget. But Giacconi says that earlier antagonism between the institute and NASA has now disappeared, as "they recognize a competent group when they see one". Independent consultants agree that the institute has performed impressively in a task whose immensity was not fully realized at the outset. One example: software for the telescope is expected to be 1.5 million lines of code, 10 per cent of that thought necessary for the Strategic Defense Initiative.

Much of the work outside the basic control system has been done in-house at STSI, which is managed independently of NASA. STSI is particularly proud of its guidestar selection catalogue, which contains the coordinates of 40 million celestial sources to unprecedented accuracy. Giacconi claims that running a project at a single institute is often more cost-effective than use of external contractors, and criticizes NASA for wanting to contract out development of the telescope's embryonic data archiving system at a cost he estimates at \$20 million. Giacconi claims STSI can develop a basic but operable system for \$0.5 million, and unfavourably compares NASA's decision to the procurement style of the Department of Defense. But he believes STSI has proved itself and speculates that it will be in business in 20 years' time "for son of the Hubble Space Telescope". **Tim Beardsley**

Sequencing the human genome

SIR—The prospect of sequencing the entire human genome (*Nature* 322, 11; 1986) raises a number of questions, some answers to which we wish to suggest.

The first is "why?". After all, not only do we not have any idea what we would find in such an alphabetic morass, but we do not even know enough about genomic structure to know what to look for. The fact that such a bizarre project may be "technically feasible" is hardly a justification. It is as technically feasible to excavate the entire country of Kenya to a uniform depth of six metres for hominid fossils, or to determine the wiring diagram of all the synaptic connections in the human brain; but any biologists who proposed such projects would doubtless be obliged to carry them out in a padded laboratory. Similarly, sequencing the genome would be about as useful as translating the complete works of Shakespeare into cuneiform, but not quite as feasible, or as easy to interpret.

A better reason is that such a major endeavour would occupy every living molecular biologist, regardless of competency, for several years, thus solving the growing unemployment crisis. The social consequences of keeping molecular biologists off the streets, where they might develop into thugs, ruffians, land-fraud swindlers, or worse, are obvious. Alternative suggestions, such as obtaining a genetic map of humans using restriction fragment length polymorphisms, suffer in that they would employ clinicians and (far worse) population geneticists and anthropologists. Any sense of social responsibility dictates keeping these latter groups on the streets where they belong. Similarly, all attempts to limit the project to sequencing only a single small chromosome must be resisted, as this would not be sufficiently labour-intensive to employ enough molecular biologists.

Second, whose genome gets sequenced? As both X and Y chromosome sequences are required, women must be excluded from consideration. A haploid set is required as diploids often show an embarrassing amount of variation between homologues. The extraction of such a set will prove difficult, given the scarcity of recorded haploids in human history. As molecular biologists generally ignore any variability within a population, the individual whose haploid genome is chosen will provide the genetic benchmark against which deviants are determined. Much care must be taken in the selection of the exemplary individual to serve as this ultimate genetic role-model; it would be most embarrassing to throw out the first 500,000 kilobases and have to start resequencing a new individual if some character flaw is discovered late in

the project.

However, the recent success of obtaining DNA from both an extinct species (*Nature* 312, 282; 1984) and a mummy (*Nature* 314, 644; 1985) suggests the possibility of using a venerable deceased human for a subject. This is quite in keeping with the spirit of the proposed sequencing project, as much of its justification involves the creation of new technologies. We would like therefore to be the first to suggest that the genome of the father of modern biology, Charles Darwin, be the one sequenced. We leave it to our English colleagues to arrange to have his remains exhumed from Westminster Abbey for the honour.

Unfortunately, it would hardly honour Darwin's memory, as the conceived sequencing project violates one of the most fundamental principles of modern biology: that species consist of variable populations of organisms. Alas, every dog has his day, and if the molecular vulgarians have theirs, "the" genome of "the" human will be sequenced, gel by acrylamide gel.

And what is to be done, finally, when this sequence is obtained? Perhaps the scientific community can enlist David Wolper to stage a worldwide tribute to the genome, culminating with every person on Earth symbolizing a nucleotide and joining hands to form all 22 autosomes and both sex chromosomes. It would be a fitting finale to a grand scientific project.

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Special relativity

SIR—Aspden¹, Psimopoulos and Theocharis² discuss the need for a Michelson-Morley type test in space and raise interesting points about the effects of standing waves in rotating and translational motion of optical apparatus. Some time ago, I carried out a relevant investigation using a special standing-wave sensor manufactured by the General Electric Co.³ This photoelectric sensor incorporates a photomultiplier tube through which a laser beam can pass to be reflected back on itself by a mirror. This allows the device to scan translationally along the standing wave set up by the interference in the beam. The experiment shows that the spacing between nodes in the standing wave set up by two oppositely-directed light rays from the same laser source is a function of the orientation of the apparatus.

The forced optical condition assuring light speed isotropy as suggested by Aspden is not supported by the experiment, and initial indications are that the beam modulation pattern is attributable to the Earth's motion through space at cosmic speeds commensurate with those found from the isotropy assumption of 3K cosmic background radiation. In effect, it appears that in the standing-wave conditions, the waves move at different speeds in opposite directions relative to the apparatus and, as their frequencies are the same, they present different wavelengths in the two directions and so affect the nodal spacing.

A detailed report on the experiment is available prior to eventual formal publication. Meanwhile, it is of interest to note that the optical configuration resembles that of the Sagnac experiment, the basis of the ring laser gyro technology mentioned by Aspden, Psimopoulos and Theocharis. However, the sensor scans linearly along a section of the modulated beam in a non-rotating system, rather than being at rest, as in the gyro, and sensing effects of rotation of the apparatus.

Clearly, this research will have interesting implications for the theory of relativity, as foreseen by your recent leading article⁴. It may also help us to resolve the large errors found in the global satellite positioning system. If present findings are sustained, it may not be necessary to extend the Michelson-Morley tests into outer space in order to obtain positive, as opposed to null, results in interferometric tests of linear motion.

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1. Aspden, H. *Nature* 321, 734 (1986).
2. Psimopoulos, M. & Theocharis, T. *Nature*, 321, 734 (1986).
3. Silvertooth, E.W. & Jacobs, S.F. *Applied Optics* 22, 1274 (1983).
4. Maddox, J. *Nature* 316, 209 (1985).

Science and faith

SIR—In his review of John Barrow and Frank Tipler's *The Anthropic Cosmological Principle* (*Nature* 320, 315; 1986), William H. Press comments that the authors' end " . . . is nothing less than the fusion of matters of science with matters of individual faith and belief. It has taken us a long time to separate these matters, each to its own legitimate arena in human affairs. We should not lightly allow them to become once again jumbled"

Although they are generally accepted, is it helpful to be given such delineations of "legitimate" arenas, which would seem to carry restrictive bias into the unfolding of human thought and experience? I personally find a segmented life no more attractive than a jumbled one.

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What happens on Cygnus X-3?

The notion that a periodic X-ray source may also be giving off streams of massive particles which are unknown from accelerator experiments is still, against the odds, alive.

OUTWARDLY, Cygnus X-3 is a run-of-the-mill celestial source of X-rays, first recognized as such (in 1966) by early rocket observations which have since been amply confirmed by measurements from Earth satellites. One of the most striking features of the object is that it is a regularly periodic source of X-rays with a period of 4.8 hours, which fits in well with the assumption that it is a binary star, one partner of which is a massive neutron star while the other is a less compact, more "normal", companion.

Cygnus X-3 is also distinguished by its intensity as a source of X-rays. Indeed, the source is thought to be rather more than 10,000 parsecs distant from the Solar System, a substantial fraction of the diameter of the Galaxy. But this circumstance goes only to sharpen another puzzling feature of the properties of the object, that it is a source not merely of ordinary X-rays but of X-rays so energetic that they are properly known as gamma rays. Indeed, there have been suggestions (see, for example, A.M. Hillas, *Nature* **312**, 50; 1984) that a few sources as powerful as Cygnus X-3 might be sufficient to account for the total content of cosmic rays in the Galaxy.

This leads to the most curious and unexpected feature of Cygnus X-3, the sketchy but intriguing suggestion that this very distant binary star may be the source of energetic particles whose nature is at present entirely unknown but which are nevertheless responsible for the production of mu-mesons (muons) at the surface of the Earth. The evidence is sketchy in the sense that it is urgently in need of independent verification, but it is also the kind of evidence that people cannot entirely set to one side.

Observations that suggest some connection between the X-ray star and the detection of cosmic rays at the surface of the Earth go back for several years. In 1983, for example, J. Lloyd-Evans and a group of colleagues at the University of Leeds (see *Nature* **305**, 705; 1983) showed that there is a correlation between the detection of energetic cosmic-ray showers in the atmosphere of the Earth and the phase of the supposed binary source of X-rays from Cygnus X-3. Whatever else may emerge about this strange object, two things are clear from the cosmic-ray observations: first, the object is a source of energetic cosmic rays that are detectable at the surface of the Earth; and, second, that the sheer production of

energy in the form of energetic particles, say gamma-ray photons, must be very large.

The measurements that have raised new possibilities were undertaken with the equipment installed deep underground in recent years in the hope of detecting the predicted radioactive decay of the proton. An unavoidable complication of these measurements is the background flux of energetic muons, which explains why there have recently accumulated data on the occurrence near the surface of the Earth of muons energetic enough to penetrate at least through the overburden of rock to the subterranean detectors.

The first reports that something remarkable may be afoot appeared just over a year ago (M.L. Marshak *et al.*, *Phys. Rev. Lett.* **54**, 2079; 1985), and were based on measurements of background muons at the proton-decay detector operated jointly by the University of Minnesota and the Argonne National Laboratory. What Marshak and his colleagues claimed is that muons observed deep underground correlate significantly with the phase of the distant X-ray star in its binary orbit. Supporting evidence has been produced by G. Battistoni *et al.*, based on measurements with the proton-decay equipment under Mont Blanc (*Phys. Lett.* **155B**, 465; 1985).

For the past year, the bare bones of the mystery of Cygnus X-3 have been plain for all to see. If there is indeed a correlation between the phase of Cygnus X-3 and the time of detection of energetic cosmic-ray particles of some kind at the surface of the Earth, the implication is that the propagation of some influence over the 10,000 parsecs between the double source and the Solar System must be accomplished in such a way that phase information is not lost on the journey, which in turn implies that differences of travel time over 10,000 parsecs must be a small fraction of the orbital period, a few minutes perhaps.

Since these circumstances were first appreciated, there has been a gale of speculation about the means by which the influence of Cygnus X-3 might be propagated over these distances. First, the precision of the timing implies that the speed of propagation must be a very large proportion of the velocity of light, which in turn implies that if particles are the medium by which the message is transmitted, they cannot be massive particles. Indeed, Marshak *et al.* last year gave good reasons why particles as massive as

neutrons would not meet the need, while particles as light as photons were similarly excluded on the grounds that there would have to be more of them than could possibly be consistent with other observations.

K. Ruddick (from the Minnesota group) now takes the argument further by pursuing the suggestion, originally made by Marshak *et al.*, that the intermediary between Cygnus X-3 and the Solar System is a flux of particles that are electrically neutral (so as not to be smeared out by the intragalactic magnetic field), relatively small in mass (so as to preserve the synchronicity of the events) and previously unknown. Ruddick's account of the problem appears in *Phys. Rev. Lett.* **57**, 531; 1986. The essence of his case is that light neutral particles are transmitted from Cygnus X-3 until they meet the rocks of the surface of the Earth, whereupon they are converted into other particles.

In principle, the argument is simple enough. Here is a set of data that cannot easily be explained in terms of existing theories, so why not invent a novel particle to account for what may be happening? The innovation in Ruddick's argument is that the primary novel particles (called "cygnets", which is natural enough given where they come from) do not interact directly with terrestrial nucleons to give the muons observed, but instead do so through the intermediary of a second unknown particle, necessarily more massive than the first. Ruddick argues that neither particle need have been observed in accelerator experiments.

The temptation to mock at the invention of a new particle of matter to solve each new problem in astrophysics should be firmly suppressed. By any yardstick, Cygnus X-3 is a remarkable object. Most of what people have written about it has been concerned with explaining how it may function as a source of very energetic X-rays. Several models have been advanced, most of which entail the impact of a particle beam from the neutron star on the more extended envelope of its larger companion. Until the likely spectral distribution of the energy produced by such a process is more exactly known, it will be hard to tell how strong is the case for extra particles. And, meanwhile, there is a great need that the original measurements should be repeated in such a way that people can tell more precisely how critical is the apparent synchronicity of phase.

John Maddox

Particle physics

Where now with superstrings?

from Robert Walgate

LITTLE more than a year since superstring theory, the best attempt yet at a 'theory of everything', began to drag particle theorists into its net, superstrings are still "the only game in town" for the unification of forces, including gravity, in a quantum framework. So says Steven Weinberg (University of Texas), who with Abdus Salam and Sheldon Glashow developed the first modern unification of forces, 'electroweak' theory, that combines electromagnetism and the weak interaction. Weinberg gave the summary talk at a recent high-energy physics conference*, where it was clear that superstrings have become an important part of particle physics.

The basic idea underlying superstring theory is that the Universe is a world of many more dimensions than the four now apparent (the number ten is now usually favoured) inhabited by myriad, almost infinitesimal, elastic strings. These strings, sometimes looped, charged, spinning and vibrating, interact to pull the extra (usually six) dimensions into a tiny, closed structure full of gaps and holes that is left attached, almost like hair, to the remaining still-extended four dimensions, a process termed compactification. Particular arrangements of this stringy hair, such as a string winding once through a particular kind of six-dimensional hole, then lead to the particles we see.

But the theory has its problems. One of them is its uniqueness: the meeting showed that there are at least six candidate theories, all of which seem (so far) to be self-consistent and free of 'anomalies', the hidden quantum violations of charge and energy-momentum conservation that have dogged all other attempts at unification. According to Weinberg, John Schwartz, who with Michael Green launched the first more-or-less realistic anomaly-free string theory, said at the meeting that the number of candidate theories (which differ in the number of dimensions, the exact constitution and charges of the strings) could even increase, although it could decrease again if inconsistencies are found in some versions.

Weinberg says the meeting was not perturbed by this development. All the versions of the theory may be the same in substance, each possibly being a different dynamical solution of a single underlying theory. This, according to one controversial view, could take the form of a string theory originally introduced many years ago as a model of the strong interactions: a bosonic string in 26-dimensional space-

time. Furthermore, another problem of non-uniqueness in superstring theory, the variety (thousands) of possible four-dimensional worlds it allows, is showing some signs of resolution. "The trouble with superstring theory, though this may not last, is that there don't seem to be any easy problems" says Weinberg. A theory should not be thrown away because it is difficult to use (the equations of hydrodynamics remain true even though it is almost impossible to calculate turbulent flow), but most difficult theories, including hydrodynamics, have certain key simple predictions through which they can be tested. But for superstrings "Schwartz said, and I agree, that there seem to be no decisive tests in sight". So superstrings remain with the theorists.

Weinberg points out that superstring theory has also disappointed early hopes of a natural solution of the 'cosmological constant problem', the question of how gravity behaves as if the vacuum is empty although in all unified field theories it is packed with a hornets' nest of forces and curvatures. Nevertheless the theory has had "many qualitative successes" and remains "a very beautiful theory, partly because of its logical rigidity".

Could there be experimental tests of the existence of strings? The phenomenology of the theories remains "murky", says Weinberg, precisely because of the number of ground-state 'worlds' which are still in practice indistinguishable. There may still be thousands of ground states to explore. One such exploration reported at the meeting requires a "great deal of mathematical virtuosity"; the resulting 'world' is "very encouraging, in that there are no problems like the proton decay being too fast, and the general pattern of quark and lepton masses looks vaguely reminiscent of the real world". But there are many 'worlds' yet to explore, each requiring its degree of virtuosity, and we may not be able to muster the labour to find the solution.

Although no projected experiments can bear directly on superstrings, there are nevertheless still some experiments of great interest that can be done. Next year should see the first experiments on the Fermilab Tevatron Collider, which will reach 2-TeV collision energies between protons and antiprotons, and the Stanford Linear Collider (SLC), which will be a 'factory' of Z's, the intermediate vector bosons that mediate the neutral part of the weak interaction. The prospect of these experiments, particularly those at the Tevatron, "loomed" over the conference,

says Weinberg. Although they are unlikely to reveal anything directly about superstrings, the SLC experiments should provide "exquisitely detailed" data on the decays of the Z, thus producing fine tests of the 'standard model', (the Weinberg-Salam electroweak theory plus quantum chromodynamics for the inter-quark force). Experiments, especially those with the Tevatron, "are going to settle the question of whether or not there is a vestige of supersymmetry at low energies".

This bears on string theory because superstrings are also supersymmetric, in that for every low-energy particle they create they also create a massive 'superpartner', a partner differing only in its higher mass, spin and quantum statistics. (Thus, if a particle obeys the Pauli exclusion principle, like the electron, its superpartner will not; and if a particle does not obey the exclusion principle, like the photon, its superpartner will do so.) Superpartners are useful in that they help explain why the weak interaction separates from the electromagnetic at energies of just a few hundred GeV, compared with the 10^{15} GeV or so at which electroweak forces separate from colour (the 'hierarchy problem'). However, superpartners only do the trick if their mass is sufficiently low. The Tevatron should see squarks, the superpartners of quarks, if their masses are less than 200-300 GeV, "a very plausible mass for them to have". If the Tevatron sees no superparticles, supersymmetry will lose its value in the hierarchy problem, and hence half its motivation. If the superpartners are found, on the other hand, we will know the masses and interactions of these long-dreamed-of particles, and "I can't even imagine what impact that's going to have on our models" says Weinberg. Superstring theory, however, could survive a non-discovery: the Calabi-Yau compactifications of the six extra dimensions were chosen, in part, to produce low-energy supersymmetry. It should be possible to find other classes of solution to the superstring equations that push the superpartner masses higher.

On other experimental issues, Weinberg is convinced by contributions at the meeting that there is a scientific "no-lose theorem" for results from the proposed superconducting supercollider (SSC), a 20-TeV collider now awaiting a decision on its construction from the US Department of Energy. Rigorous calculations in which space-time is approximated as a lattice (a common technique in modern quantum field theory) show that the SSC must reveal the origin of the symmetry breaking that divides electromagnetism from the weak interaction at low energies. This mechanism could either involve a vacuum full of a 'Higgs field', or a new superstrong 'technicolour' force that could lead to strong interactions between intermediate vector bosons. According to

*XXIII International Conference on High-Energy Physics, 16-23 July 1986, Berkeley, California.

this no-lose theorem, the Weinberg-Salam electroweak theory, which is a low-energy approximation invoking the Higgs mechanism, must break down to some 'new physics' at an energy that depends on the mass of the lightest observable Higgs particle. If the Higgs mass is low (100 GeV), the breakdown does not appear until 10^{19} GeV where quantum gravity enters and new physics is certain. If the Higgs mass is only 10 times higher, new physics sets in "almost immediately". In either case, the corollary for the SSC is that there will be something fundamental to observe: either Higgs at 100 GeV; or both Higgs and the new physics, possibly technicolour.

Solar neutrinos were also central to discussions at the meeting. The new 'resonance' mechanism explains the low incidence of solar neutrinos in Raymond Davis's electron neutrino detector at Homestake Mine, South Dakota in terms of a resonant, efficient conversion of solar electron neutrinos into higher-mass neutrino species in the Sun. This mechanism requires the neutrinos to have masses that turn out to be of exactly the order predicted by a wide class of unified field theories, according to Weinberg, who presented very general arguments that the mass of the heaviest neutrino should be around the square of the top quark mass divided by the grand unification scale (about 10^{15} GeV). This works out at around 10^{-3} eV, whereas the solar resonance model is tolerant of a range from 10^{-3} to 10^{-5} eV, depending on mixing angles between neutrino types. Weinberg argues, therefore, that a test of the resonance model is now "as important as anything else in particle physics". Several experiments are already at proposal stage. The best method would be to look for weak neutral current interactions of the solar neutrinos arriving at the Earth, Weinberg suggests, because all the incoming neutrinos would interact equally, the neutral current being 'democratic' in neutrino types.

If the Davis experiments, which measure charged-current interactions, are seeing few electron neutrinos because there are fewer of these particles produced in the solar core (perhaps because the temperature is lower than expected) then a neutral current experiment will see the same number. But if the threefold depression below predicted rates that Davis sees is caused by the conversion of two-thirds of the electron neutrinos to other neutrino types, then a neutral current experiment will see three times Davis's signal. Weinberg says that this experiment "may be our lucky break", the only indication of the grand unification of forces that comes down to us from 10^{15} GeV, now proton lifetimes seem too long to detect. □

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Planetology

Sulphur and volcanism on Io

from David A. Crown and Ronald Greeley

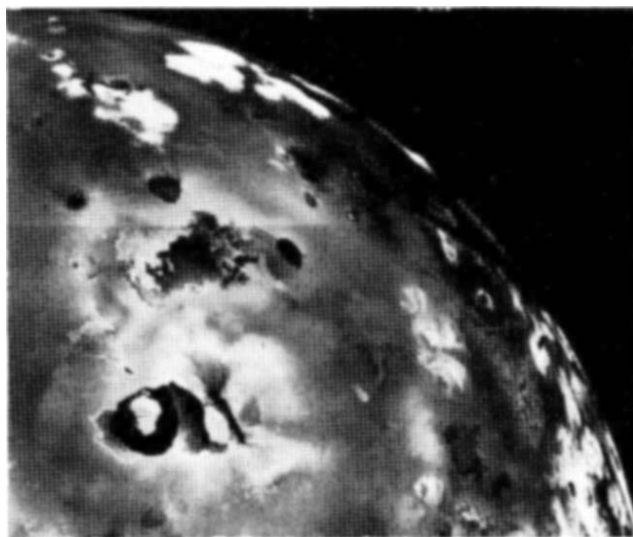
SULPHUR on Io was suggested even before Voyager 1 and 2 flew by the jovian system in 1979^{1,2}, and although its existence is now generally accepted, its precise role remains controversial. The morphology of volcanic landforms argues for a surface formed by silicate volcanism, whereas spectral data indicate a surface dominated by sulphur (see ref. 3 for review). Lunine and Stevenson⁴ recently presented a model for hot spots, regions of intense volcanism on the surface of Io, as lakes of molten sulphur.

Active volcanoes were observed on Io

into account the physics of convecting, boiling systems and the transport of vapour away from such systems. The authors suggest that the temperatures and thermal fluxes observed in the Loki Patera region of Io (see figure) are consistent with their model of a convecting sulphur lake and that the total thermal flux is consistent with the maximum that could be derived from a convecting silicate magma chamber. Their model also explains the distribution of thermal energy around Loki Patera observed during the Voyager missions and indicates that evaporation

constrains the maximum surface temperature of a sulphur lake under steady-state conditions.

Lunine and Stevenson's model provides the means to relate the surface heat flow detected by Earth-based infrared observations to changes in the temperature of a sulphur lake and variations in the output of silicate magma chambers in Io's crust. The authors estimate that if lake renewal or magma chamber lifetimes are limited to 1–100 years, then changes in the thermal output in the Loki Patera region could be detected. Continued Earth-based observations at



Voyager 1 mosaic showing the eruption of Loki (lower left). Plumes emerge from a linear black fissure approximately 200 km long. The crescent-shaped large dark patch to the left of the fissure may be a lava lake with a section of solidified crust in its interior. Other vents are seen in the image as dark, nearly circular patches surrounded by lava flows and volcanic plains. The original mosaic was processed by A.S. McEwen (Arizona State University) and the US Geological Survey.

during the Voyager 1 and 2 missions and are attributed to the dissipation of tidal energy resulting from the configuration of Io's orbit in the jovian system⁵. McEwen *et al.*⁶, using Voyager infrared interferometric spectrometer results and multi-spectral data, correlated dark calderas with areas of high heat flow. Recent experiments concerning the optical properties of sulphur are consistent with this correlation and indicate that molten sulphur on the surface of Io would be black as observed by Voyager instruments⁷.

Lunine and Stevenson explain the hot spots using a model for a convecting sulphur lake heated by an underlying silicate magma chamber. The model considers the physical and chemical processes in convective sulphur lakes, including the thermodynamic and transport properties of sulphur and the effects of some possible impurities. In addition, the model takes

infrared wavelengths are important to distinguish between: (1) high temperature (600 K) outbursts isolated from the Loki region (volcanic eruptions); (2) independent changes in warm or hot components in the Loki region (the warm component, the result of the latent heat released on condensation of evaporative sulphur in the atmosphere surrounding a sulphur lake, and the hot component caused by the heat released by convecting molten sulphur); and (3) changes in sulphur lake temperature and the corresponding evaporative sulphur flux. Consequently, Earth-based monitoring could provide important information about Io's thermal output and volcanic activity.

Lunine and Stevenson also consider the effects of impurities such as sodium polysulphides. If the base of a sulphur lake became enriched in sodium polysulphides, convection of this material might

occur and a liquid polysulphide melt could be exposed at the surface on evaporation of the overlying sulphur. Sodium polysulphide lakes or remnants of such lakes may occur in the Loki Patera region and elsewhere on Io and could be distinguished from convecting sulphur lakes using infrared observations at several wavelengths. Lunine and Stevenson suggest that such polysulphide lakes could be the source for the sodium-enriched material in the Io torus.

Although many assumptions are involved, Lunine and Stevenson's rigorous application of theory to both Earth-based and Voyager observations demonstrates the importance of continued monitoring of Io from Earth and is an example of the type and quality of work necessary to plan future observations effectively. In particular, the sulphur lake model can be

evaluated during the Galileo mission in the 1990s. Lunine and Stevenson suggest that during the cruise phase of each orbit, thermal measurements, including those from the night side of Io, could be used to characterize and to detect variations in hot-spot activity. □

1. Fanale, F.P., Johnson, T.V. & Matson, D.L. *Science* **186**, 922 (1974).
2. Wamsteker, W., Kroes, R.L. & Fountain, J.A. *Icarus* **23**, 417 (1974).
3. Nash, D.B., Carr, M.H., Gradie, J., Hunten, D.M. & Yoder, C.F. in *Satellites* (eds Burns, J.A. & Mathews, M.S.) 629 (University of Arizona Press, Tucson, 1986).
4. Lunine, J.I. & Stevenson, D.J., *Icarus* **64**, 345 (1985).
5. Peale, S.J., Cassen, P. & Reynolds, R.T. *Science* **203**, 892 (1979).
6. McEwen, A.S., Matson, D.L., Johnson, T.V. & Soderblom, L.A. *J. geophys. Res.* **90**, 12345 (1985).
7. Sasson, R., Wright, R., Arakawa, E.T., Khare, B.N. & Sagan, C. *Icarus* **64**, 368 (1985).

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Plant viruses

New threats to crops from changing farming practices

from Robert Coutts

IN THE 25 years since it was first suggested that root-inhabiting fungi act as vectors of plant viruses¹, many such viruses have been identified and some characterized in detail (see ref. 2 for a recent review). The importance of these viruses as plant pathogens in the United Kingdom was largely ignored until recent changes in agricultural practice as well as the geographical proximity of the fungus-vectored viral agent of rhizomania, beet necrotic yellow-vein virus, renewed interest³. Will an increased awareness of the incidence of soil-borne viruses or further changes in agricultural practice result in the appearance of further 'new' viruses and their associated diseases?

Of immediate concern to farmers in the United Kingdom is the spread of barley yellow mosaic virus^{4,5} carried by zoospores of the primitive plasmodiophoromycete *Polymyxa graminis* Ledingham, that affects winter barley. (Beet necrotic

yellow-vein virus is transmitted by another member of this group, *P. betae* Keskin.) The barley virus is widespread, causing variable reductions in grain yield, because farmers now grow winter malting barley as a profitable spring crop. Local soil conditions, such as low temperature and high humidity, are ideal for fungus transmission of the virus and the development of symptoms. As temperatures rise in the spring the symptoms of the disease fade but infected plants remain stunted.

After transmitting the virus to the plants, the zoospores develop into cystosori which are found in the roots and which can remain viable for extended periods (see figure). This feature of the fungal life-cycle ensures perpetuation of the fungus and virus in areas of intensive winter barley production. Recent reports of the incidence of more than one naturally occurring strain of the virus⁶ suggests any inbred resistance may not be durable.

In the long term, biological control of *Polymyxa* with other rhizosphere-inhabiting microorganisms may be possible. For example, cystosori of *P. betae* can be parasitized and completely degraded by *Trichoderma harzianum* Rifai *in vitro*⁷, offering hope for the control of rhizomania, the sugar-beet disease that causes havoc in Europe. This disease and the causal agent, beet necrotic yellow-vein virus, have recently been diagnosed in France, the Netherlands, Belgium and Luxembourg. Import of sugar-beet stocklings from Europe to the United Kingdom is now banned⁸.

P. graminis and *P. betae* can be differ-

entiated only by host specificity, and the spread and transmission of the beet and barley viruses described here appears similar. A recent survey⁸ failed to find the beet virus or the disease in the United Kingdom but does reveal the presence of a morphologically similar but serologically distinct virus⁸. This virus, or one very similar to it, had been isolated previously from beet plants in Norfolk displaying root-symptoms of the Barney patch disorder. The virus, provisionally named beet soil-borne virus, is also apparently transmitted by *P. betae* but its pathogenic effects, if any, are unknown. The catastrophic effects of rhizomania are well documented, with the sugar content of infected beets reduced to 10 per cent or less of that found in uninfected controls³.

Changes in horticultural practice have increased the incidence of another group of fungus-borne viruses whose appearance coincides with the increased growing of plants in soil-free environments, where plants are cultivated on solid-support matrices and fed with liquid nutrients. Nutrient-film growing is popular with growers for several reasons, such as its application to greenhouse crops, ease of maintenance, monitoring of nutrient supply, quality control and cost. But two outbreaks of disease in the last six years, lettuce big vein virus⁹ and necrotic spot virus in cucumber¹⁰ seem to be a direct result of nutrient-film growing.

The lettuce and cucumber viruses are transmitted by zoospores of the aquatic phycomycetes *Oplidium brassicae* and *O. radicale*, respectively. However dissimilar from the situation with the plasmodiophoraceous fungi, chemical control of phycomycete fungi and the diseases they transmit appears feasible — the two outbreaks of lettuce and cucumber viruses were successfully controlled by continuous application of a non-ionic liquid surfactant that killed the transmitting zoospores^{9,10}.

It is unlikely that the viruses I have described in this article are new to the United Kingdom. It is more plausible that interactions between the viruses, vectors and hosts are now acting under the correct environmental conditions for infection to result. □

1. Teakle, D.S. *Nature* **188**, 431 (1960).
2. Brunt, A.A. & Shikata, E. in *The Rod-shaped Plant Viruses* (ed. van Regenmortel, M.H.V.) (Plenum, New York, in the press).
3. Dunning, R.A. *Brit. Sugar Beet Rev.* **52**, 47 (1984).
4. Adams, M.J. *Rothamsted A. Rep. Part I* 130 (1984).
5. Hill, S.A. *Plant Path.* **29**, 197 (1980).
6. Ehlers, U. & Paul, H.-L. *J. Phytopath.* **115**, 294 (1986).
7. D'Ambra, V. & Muttio, S. *J. Phytopath.* **115**, 61 (1986).
8. Henry, C.M., Jones, R.A.C. & Coutts, R.H.A. *Plant Path.* (in the press).
9. Tomlinson, J.A. & Faithfull, E.M. *Ann. Appl. Biol.* **93**, 13 (1979).
10. Tomlinson, J.A. & Thomas, B.J. *Ann. Appl. Biol.* **108**, 71 (1986).

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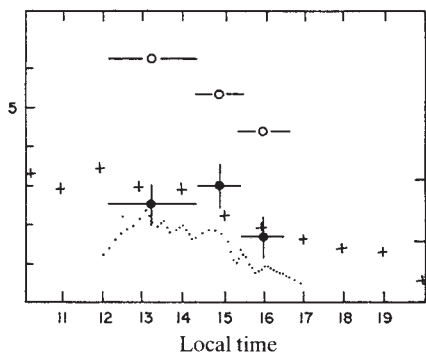
Infected barley root cell full of resting spores (cystosori) of *P. graminis*, the vector of barley yellow mosaic virus. (Photograph courtesy of E.J.F. Roberts and K.G. Laing.)

Atmospheric chemistry

Hunting tropospheric OH ions

from D. Perner

Is the self-cleansing capacity of the troposphere still adequate to cope with the ever-increasing amounts of emitted gases? Or could unchecked anthropogenic emissions eventually make populated areas uninhabitable? To measure accurately concentrations of the free hydroxyl radical (OH), generally regarded as the main agent for direct oxidation and for removal of substances from the atmosphere, has been a major challenge¹. The article by Hard and co-workers on page 617 of this issue⁵ describes measurements obtained by a promising new spectroscopic technique which should allow a test of whether



Comparison of the diurnal variation of measured OH concentrations $\times 10^6 \text{ ml}^{-1}$ (left-hand scale) observed by optical absorption⁸ on 19 May 1983 at Deuselbach (black circles), and by laser-induced fluorescence⁵ on 18 June 1985 at Portland (crosses). Also shown are the O₃ photolysis frequency $\times 10^{-5} \text{ s}^{-1}$ (right-hand scale; dotted line) and the calculated OH concentration (open circles), both for Deuselbach⁸.

our understanding of atmospheric photochemistry is consistent with measured OH concentrations.

It is only since the early 1970s that the central role of the OH free radical in the troposphere has become apparent. In 1969 Weinstock⁶ ascribed the strong variability of carbon monoxide in the troposphere to the action of OH radicals, but he could not identify their source. Two years later, Levy⁷ found that not all of the Sun's ultraviolet light, which produces excited oxygen atoms from ambient ozone, was filtered out by the stratosphere. Because this excited oxygen is the primary intermediate for OH formation, an appreciable source of OH therefore exists in the troposphere.

Although highly labile, OH does not react with any of the major atmospheric components, so it can exert its full oxidative power on trace gases such as methane, carbon monoxide and methyl chloride, which are eventually converted into carbon dioxide, water and other

stable products that are washed out of the atmosphere. This process has prevented trace compounds from building up in the atmosphere over the millennia. OH is important because it is regenerated in these oxidation processes: in the free atmosphere, typically up to five oxidation reactions are initiated by OH before it is scavenged. Despite this amplification of OH, fast chemical reactions keep its ambient concentration quite low, to about a few million molecules per millimetre in sunlit air. It is also commonly accepted that the concentrations at night are considerably below those in the daytime.

Several *in situ* techniques for measuring OH concentrations are presently in use. Radiolabelled products, such as ¹⁴CO₂ formed from ¹⁴CO by the OH reaction, can be measured with high-sensitivity or spectroscopic methods to probe the OH concentrations directly^{1,3}. Hard *et al.*⁵ apply laser-induced fluorescence at low pressure in a manner that excludes ambient light from the detector. In this way difficulties previously found using this technique are circumvented and the instrument can be converted easily for aircraft measurements.

The midday OH concentrations found in Portland by Hard *et al.*⁵ can be compared with similar observations in Deuselbach by my own group obtained by long-path optical absorption (see figure). Whereas in Deuselbach the OH signal disappears at 1700 h, the OH in Portland

extends fairly late into the evening and, surprisingly, is found at night. The reason for this unexpected and interesting observation is unknown. Other types of data from Portland are not detailed enough to calculate a model of the OH distribution. In contrast, other types of data from Deuselbach predict much higher OH concentrations than are found experimentally there. Thus the theoretical description at Deuselbach cannot be applied.

I still believe that direct evidence for free tropospheric OH, preferably by spectroscopy, is needed. Simultaneous measurements of all species and parameters in the OH photochemical cycle, together with the OH measurements, are now a prerequisite to test our atmospheric theory. Investigations of various air masses should be made to represent the whole of the troposphere, and then we could distinguish between the importance of homogeneous gas-phase chemistry and heterogeneous processes taking place at the surface of aerosols or in droplets. Only then shall we learn whether the observed increase in concentration of climate-effective gases like methane is caused in part by a decline in atmospheric self-cleansing by OH. □

1. Davis, L.J. *et al.* *J. geophys. Res.* **90**, 12835 (1985).
2. Huebler, G., Perner, D., Platt, U., Toennissen, A., & Ehhalt, D. *J. geophys. Res.* **89**, 1309 (1984).
3. Rodgers, M.O., Bradshaw, J.D., Sandholm, S.T., KeSheng, S., & Davis, D.D. *J. geophys. Res.* **90**, 12819 (1985).
4. Sheppard, I.C., Hardy, R.J., & Hopper, F. *Antarctic J.* **206** (1982).
5. Hard, T.M. *et al.* *Nature* **322**, 617 (1986).
6. Weinstock, B. *Science* **166**, 224 (1969).
7. Levy, H. *Science* **173**, 141 (1971).
8. Perner, D. *et al.* *J. atm. Chem.* (submitted).

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Archaeology

Japanese agricultural beginnings

from Gina L. Barnes

AGRICULTURE in the Japanese islands was long thought to begin with the importation of wet rice technology from the continent at around 300 BC, an event taken to mark the transition from a hunting and gathering economy in the postglacial Jomon period to a period of settled agricultural communities. But this traditional view has been thoroughly overturned in the past ten years with discovery of the remains of many cultivated plants—including rice—from Jomon sites. The recovery of buckwheat pollen from the Ubuka bog in southwestern Japan, reported on page 632 of this issue, provides the strikingly early date of $6,600 \pm 75$ years before present (BP) for buckwheat cultivation, paralleled only by the finds of beans and gourd at the Torihama site which are dated to 5,000–

3,500 BC. If these data become widely accepted, they may lead to a substantial revision of our ideas about the pattern of human settlement during this period.

Models allowing flexibility in determining the role of plants in the different regional and temporal complexes of the Jomon period have already been developed^{3,4} and a major advance has been the correlation of population densities with forest and fishery zones^{5,7}. This correlation indicates that the population occupying the southwestern evergreen oak-laurel forest at low density was less dependent on hunted animals—scarce in such a forest regime—and therefore more dependent on plant foods. The abundant floral remains at Torihama from the early Jomon period support this idea and

Akazawa believes this background accounts for the rapid spread of rice agriculture through western Japan once it was introduced from the continent towards the end of the Jomon period⁷. His corollary is that the populations of coastal north-eastern Japan that had highly successful fishing economies resisted the introduction of rice technology.

The beginnings of plant cultivation in western Japan are based on the concept of the 'Laurilignosa culture' developed by Nakao (see ref. 8). The composition of the evergreen oak-laurel (Laurilignosa) forest that became established around 7,000 BC was part of a greater continental forest zone occupied by people practising 'slash and burn' agriculture. The extension of this economy into the Japanese portion of this forest zone is held by numerous Jomon archaeologists who have interpreted charred layers in excavation stratigraphy as the remains of burned-forest fields. Although slash and burn systems that include systematic fallowing cannot yet be distinguished stratigraphically from simple forest-clearing activities using fire, Tsukada⁸ not only shows the sudden appearance of charcoal fragments in the Ubuka bog cores but he also provides palynological evidence for the crops being grown — in this case buckwheat.

Most evidence for Jomon cultivation has been in the form of macroremains or seeds (for example, see ref. 9). Methods of identifying cultivated plants from fossil pollen and plant phytoliths¹⁰ have only recently been refined to the point of credibility, and palynological evidence is still viewed with scepticism by many Jomon archaeologists. Tsukada's method of filtering out large pollen grains in order to count the percentage occurrence of buckwheat is still unpublished, and his identifications of millet pollen remain tentative¹¹. Moreover, the method used by Japanese palynologists to identify rice pollen (see ref. 12) is not yet complete.

Nakamura's method combines transmission electron microscopy and phase-contrast microscopy, by which three groups of grass pollen are distinguished according to their surface morphologies. Nakamura states that this is at best a method for tentative identification, for although rice (*Oryza*) can be distinguished from pollen of the other types, it cannot be

distinguished from *Agropyron* (Graminae) and *Arundinaria* (Bambusoideae), both of which grow in similar ecological niches as *Oryza*.

Nevertheless, Nakamura and colleagues have identified rice pollen in Jomon sites dating to 1,200, 1,000 and 900 BC (for example, ref. 11). Japanese archaeologists are reluctant to accept these early dates, however, and still consider the appearance of rice-growing in Japan at around 400 BC on the basis of archaeological evidence of field systems and rice macroremains.

Clearly, research on the different crops and their cultivation systems in the Jomon period of Japan is extremely controver-

sial, with various disciplines offering differing forms of evidence and interpretations. The debates may lead to substantial revision of ideas on the Jomon, once thought to be a very stable and long period of foraging, and may reveal a number of adaptive strategies and mixed subsistence economies existing through time and space¹⁰. These results are important not only for Japanese prehistory but also for understanding changing human-plant relationships throughout the world in the postglacial period. □

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Invertebrate neurobiology

Catching up with peptides

from Mark Tyrer

OUR knowledge of the structure of most bioactive peptides in invertebrates is too scanty to exploit the experimental advantages provided by their large, accessible central neurones. If we could use these advantages to the full, invertebrate neurones could provide model systems for testing at the cellular level some intriguing hypotheses about peptide function in the vertebrate central nervous system. Paradoxically, the small size of the invertebrate nervous system, which makes it so attractive for physiology, is an obstacle to isolating and sequencing native peptides. At a recent meeting* it was clear that many invertebrate peptides are now beginning to yield to developments in high-pressure liquid chromatography, amino-acid sequencing and recombinant DNA technology. These developments mean that the small amounts of nervous tissue available from invertebrates are becoming less of a problem and the distribution and function of some native peptides are now being studied in detail.

The structures of several invertebrate peptides have now been deduced, including the crustacean cardioactive peptide CCAP, a nonapeptide different from any previously described peptide in that it has two cysteine residues which are thought to form a disulphide bridge, producing a hairpin shape (J. Stangier, Bonn). A neuropeptide from the sea anemone *Anthopleura* and three new peptides from *Aplysia* have also been sequenced. Insect diuretic hormone has been partially sequenced and others, such as arginine-vasopressin-like insect diuretic hormone (AVP-DH); a moth pheromone-release-stimulating peptide; and a crustacean moult-inhibiting hormone, are on the way.

The purification of prohormones for invertebrate peptides is particularly exciting. Because several peptides are commonly cleaved from one prohormone, cleavage products may include, in addition to known peptides, some which have not yet been identified. The prohormones for locust AVP-DH, locust adipokinetic hormone, *Aplysia* pro-FMRF amide (Phe-Met-Arg-Phe-NH₂, or Femeramide) and snail ovulation hormone have been isolated. The amino-acid sequence of the FMRF-amide precursor (R. Scheller, Stanford University) has been obtained by recombinant DNA techniques. It contains multiple copies of FMRF amide, a single copy of Phe-Leu-Arg-Phe-NH₂ (FLRF amide) and three regions that have some amino acids in common with mammalian corticotrophin, melatonin-stimulating hormone and corticotrophin-like internal peptide.

Several peptides produce membrane changes in target cells in preparations such as the motor nerve net of the coelenterate *Polyorchis* (A.N. Spencer, Edmonton), and central neurones in *Aplysia* (Scheller) and *Helix* (G. Cottrell and N.W. Davis, St Andrews). In leg muscle cells in locusts and cockroaches, M. O'Shea (Geneva) demonstrated the value of invertebrate preparations for examining the interactions of peptides with other transmitters. All the neurones innervating the muscle have been identified and are accessible to intracellular recording and biochemical analysis. The classical transmitter L-glutamate and the peptide proctolin co-exist in the slow motor neurone to the coxal depressor muscle. Proctolin released from this neurone amplifies the twitch responses of the muscle to co-released glutamate. At the same time proctolin causes a direct slow contraction of the coxal depressor muscle that seems

1. Rowley-Conwy, P. *World Archaeol.* **16**, 28 (1984).
2. Tsukada, M., Sugita, S. & Tsukada, Y. *Nature* **322**, 632 (1986).
3. Nakao, S. *Dominion* **13**, 6 (1977).
4. Nishida, M. *J. anthrop. Archaeol.* **2**, 305 (1983).
5. Koyama, S. *Senri ethnol. Stud.* **2**, 1 (1978).
6. Akazawa, T. *Senri ethnol. Stud.* **9**, 1 (1981).
7. Akazawa, T. *Univ. Mus. Univ. Tokyo. Bull.* **27**, 73 (1986).
8. Tsukada, M. *Kodansha Encyclopedia of Japan* **5**, 92: 7, 177 (1983).
9. Kotani, Y. *Senri ethnol. Stud.* **9**, 201 (1981).
10. Crawford, G. *Anthrop Pap.* No. 73 (University of Michigan, 1983).
11. Tsukada, M. in *Windows on the Japanese Past: studies in archaeology* (eds Pearson, R. et al.) (University of Michigan, in the press).
12. Nakamura, J. *Quat. Res.* **13**, 187 (1974).

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to result from release of intracellular calcium. Octopamine released from another motor neurone also amplifies glutamate-induced contractions in the extensor tibialis of the insect. It is probable that cyclic AMP is the second messenger for octopamine and that inositol phosphates mediate the action of proctolin.

Because it has been so difficult to purify invertebrate bioactive peptides many researchers have used antibodies directed against vertebrate peptides for immunohistochemistry on invertebrate systems. Immunoreactivity to vertebrate neuro-peptides can provide useful anatomical information; it can also be used to purify a native peptide, either as an effective assay (H. Duve and A. Thorpe, Queen Mary College, London; J. Proux *et al.*, Bordeaux) or as a useful ligand for immunofluorescence (Proux *et al.*).

The importance of peptides for signalling in nervous systems is becoming increasingly apparent. Most neurones, both in invertebrates and vertebrates, probably contain peptides as well as classical neurotransmitters. Whereas classical transmitters are released at specialized synaptic

junctions, exocytosis of granule-containing peptides often occurs at unspecialized regions of the plasmalemma. This was first recognized in the central nervous system of the earthworm (D. Golding, Newcastle) and is now widely seen if tannic acid (which appears to prevent immediate dispersion of the exocytosed granule) is used in fixing specimens for electron microscopy. Both Golding and J. Joosse (Amsterdam) suggested that peptide transmission is the more primitive method of signalling; classical synaptic transmission could be a specialized development to produce a highly efficient but more inflexible means of information transfer. Joosse argued further that peptides, because of their close relationship to the genome, can be viably altered by any change in the DNA and hence can easily alter their function. Thus, although they may be a less precise method of signalling than classical transmitters, peptides could be involved in adaptation of individuals or populations to changes in the environment. □

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Gene regulation

Fingers and DNA half-turns

from Stephen C. Harrison

AT first sight the DNA sequences from eukaryotic transcriptional control regions give an impression of overwhelming complexity. The precedent of prokaryotic control suggested simplification should come from structural analysis of the proteins that recognize these sequences; and indeed it seems that understanding of transcriptional regulation of 5S RNA genes in the toad *Xenopus laevis* has now reached this stage¹. At least three protein factors are required for transcription of these genes by RNA polymerase III. One of them, transcription factor IIIA (TFIIIA), has in its amino-acid sequence an approximately 30-residue motif repeated nine times, probably corresponding to nine zinc-binding sites². In the 4 July issue of *Cell*, Rhodes and Klug³ point out a corresponding repeat in the sequence of DNA bound by TFIIIA and suggest that it has some unusual structural features. Moreover, they identify similar repeats of approximately 5 base pairs (bp) in control regions of other genes, including some transcribed by RNA polymerase II. The possibility that these DNA sequences have overall structural features important for recognition by transcription factors is also raised directly by the report of McCall *et al.* on page 661 of this issue⁴. They describe the crystal structure of a double-stranded nonadexynucleotide that corresponds to the tightest binding

part of the sequence recognized by TFIIIA and show that it has an A-type rather than a B-type conformation.

There are two multigene families for 5S RNA in *Xenopus*, differentially expressed in oocytes and in somatic cells, respectively¹. A single molecule of TFIIIA binds to an approximately 50-bp control region in the middle of the genes, and this interaction is essential for the formation of a transcription complex that directs accurate initiation by RNA polymerase III^{5,6}. The site has been defined by deletion mapping⁷, by DNase I protection and by binding interference from base methylation or phosphate ethylation⁸. TFIIIA also forms a 7S complex with the 5S transcript, and immature oocytes store a vast number of 5S RNA molecules in the form of this 1:1 complex⁹. Proteolytic cleavage of 7S particles divides the TFIIIA polypeptide, which has a relative molecular mass of 40,000 (40K), into two domains — a 30K amino-terminal domain with full binding activity and a 10K carboxy-terminal domain essential for transcriptional activation¹⁰. One amino-terminal domain binds to 50 bp of DNA, and Smith *et al.*¹⁰ postulated a very extended structure for the protein. Miller *et al.*² show that the complementary DNA-derived amino-acid sequence of the 30K domain has nine units of an approximately 30-residue, tandemly repeated motif. Each copy of the motif

contains two cysteine and two histidine residues in conserved positions. The 7S TFIIIA/5S RNA complex contains Zn, and TFIIIA requires this ion for DNA binding. In proteins, Zn is generally tetrahedrally coordinated, often by cysteine or histidine side chains, and indeed Miller *et al.* find sufficient Zn in the 7S particles to propose that each 30-residue motif constitutes a Zn-binding 'finger'. The 30K, DNA-binding domain of TFIIIA thus appears to be a nine-finger repeated structure.

Sequence comparisons indicate the presence of similar motifs in other proteins that interact with DNA or RNA^{12,13}. These include the small RNA-binding protein encoded in the *gag* genes of retroviruses; the E1A gene-activating protein of adenoviruses; and the large-T antigens of papovaviruses¹². An even more extensive similarity has been found between TFIIIA and the product of the *Drosophila* developmental, regulatory *Krüppel* locus¹³. Thus TFIIIA probably represents a class of structures, with a metal-binding site as a significant element of organization.

If TFIIIA contains a nine-finger repeat, is there a corresponding repeat in the 50-bp intragenic control region to which it binds? Rhodes and Klug³ present evidence for such a repeat in the DNA itself, not obvious from casual inspection, but revealed by quantitative study of DNase I cleavage patterns and by mathematical analysis of the sequence. Cleavage of defined restriction fragments at different positions by DNase I is strikingly non-uniform, suggesting strong dependence of the cleavage rate on details of local DNA backbone conformation. DNase I binds across the minor groove, cutting each strand separately¹⁴. Activity may be influenced by the width of the minor groove or the orientation of phosphates¹⁴. A periodicity in the probability of DNase I cleavage along a given strand therefore probably reflects a periodicity in the DNA structure.

Rhodes and Klug's Fourier analysis of DNase I cleavage patterns of the TFIIIA binding sequence shows a periodicity of about 5.7 nucleotides. The periodicity is characteristic of both strands, with a relative stagger of about 1, rather than 2 or 3 as generally observed for B-type DNA. The smaller stagger suggests that base pairs are tilted, as in A-form DNA, so that phosphodiester bonds staggered by only one step lie nearly under each other across the minor groove¹⁴. Recognition of an A-form structure would of course be convenient for a protein that must bind specifically to RNA as well. Rhodes and Klug³ also observe an approximately 5.6-base periodicity in the sequence of the TFIIIA binding site by focusing on the distribution of guanines. Fourier analysis confirms the significance of this repeat, which is not characteristic of randomly

selected nucleosome core DNA. The total of nine such repeats in the internal control region of the 5S RNA corresponds precisely to the nine putative binding fingers in TFIIIA, each of which would tend to make the same sort of contact with DNA.

The crystal structure of a 9-bp double-stranded deoxyribonucleotide with the sequence d(GGATGGGAG) representing base pairs 81–89 of the TFIIIA binding site, is reported in this issue⁴. It supports the notion that the 11-bp repeat (2×5.7 in the DNase I analysis or 2×5.6 in the sequence periodicity) and the 1-bp DNase I cleavage stagger observed by Rhodes and Klug³ do reflect an A-type conformation. The structure has been determined and refined at 3.0 Å resolution. It has a mean twist of 31.3°, corresponding to a loosely defined helical repeat of 11.5 bp, and an overall configuration rather close to the model derived from fibre-diffraction studies of A' RNA¹⁵. The major groove in A-DNA is narrower and deeper than in B-DNA, but the structure determined by McCall *et al.*⁴ shows that there is room for a loop of polypeptide to fit — for example, the twisted β -ribbon suggested as a model for a single TFIIIA finger⁷. McCall *et al.* propose that the GpG steps are the origin of the A-form structure for this fragment. In all structures with GpG/CpC observed to date, the five-membered ring of one guanine base lies stacked on the six-membered ring of its neighbour, an apparently favourable base-to-base overlap that is characteristic of A-form double helices.

The significant conformational plasticity of DNA gives rise to a caveat, however, which McCall *et al.* discuss. The A structure often occurs in crystals of oligonucleotides — perhaps more often than for the corresponding sequences in solution — and the relationship between solution and crystal conformations is not completely understood. In particular there are unknown influences of end-effects; of electrostatic interaction; and of solvent dielectric constant (the crystals studied by McCall *et al.* were prepared in 20 per cent methylpentanediol). Nonetheless, it is probably safe to assume that a structure found in a highly hydrated crystal will not differ drastically in free energy from whatever configuration dominates in solution and that the crystal structure represents an important component of any equilibrium distribution.

The significance of the half-turn repeat discovered in the internal control region of 5S RNA genes is enhanced by indication of similar repeats in other control elements. Rhodes and Klug³ present evidence for a repeated motif of pairs of guanines within the split transfer-RNA gene promoter of various eukaryotes and within homologous positions of Alu sequences. They also observed that the so-called 21-bp repeats in Simian virus 40

(ref. 16), three tandem elements known to bind transcription factor Sp1 (ref. 17), have an effective 5 1/4-bp periodicity that is particularly striking when the positions of guanines are analysed. They propose that many factors directing transcription of transfer RNA genes by RNA polymerase III, as well as Sp1, which directs transcription by RNA polymerase II, contain TFIIIA-like fingers that interact with half-turn sequences of DNA.

The modular character of this proposed one-finger/one-half-turn correspondence is its most striking feature. Because there is good evidence that TFIIIA binds to one side of the DNA helix¹⁸, it is plausible that the fingers project alternately into the major groove on either side of the double helix. Evolutionary schemes can be constructed that would add modules and modify local binding affinities if such a linear relationship between protein modules and DNA half-turns indeed turns out to be true. The position of introns in the TFIIIA, most of which lie just between the putative fingers, is certainly consistent with these ideas¹⁹.

Such an evolutionary history does not, however, imply that the repeat structures in TFIIIA bind independently. Indeed, analysis of binding to deletion mutants in the TFIIIA site, preferential proteolytic cleavage patterns and fragment-binding experiments all suggest that the amino-terminal part of TFIIIA, the 20K segment that corresponds to the first five repeats, binds as a unit^{7,10}. Moreover its binding is required for binding the next part of the protein (the remaining four repeats). The nine fingers might be grouped into two hands.

Several bacterial control proteins have a different but equally striking modular character²⁰. The recurring module is a 20-residue helix-turn-helix elbow first identified in the *cro* repressor protein of bacteriophage lambda²¹ and in the catabolite activator protein of *Escherichia coli*²². This structure has so far always been found embedded in a larger DNA-binding domain (60–90 residues) of a dimeric or tetrameric protein. The 2-fold symmetry of the protein corresponds to an approximate 2-fold symmetry in the DNA sequence with which it interacts. Contacts to the DNA backbone, made by residues at various positions in the binding domain, position the helix-turn-helix module to interact with bases in the major groove²³. In most cases, the points of major-groove interaction by the two members of the dimer are one turn apart, although in at least one case, the interaction may be two turns apart²⁴. The repressor of bacteriophage 434, for which the crystal structure of a complex with operator is known, exhibits a specificity for B-form DNA²⁵. The same seems to be true for other helix-turn-helix-containing proteins. This sort of modularity found in the prokaryotic

repressors and activators is different from that proposed for TFIIIA, most simply because it involves a nearly precise 2-fold symmetry rather than approximate translational symmetry, and structurally because it involves identical protein subunits associated as dimers rather than homologous units concatenated in a single polypeptide chain.

TFIIIA and many of the prokaryotic repressors and activators have one significant structural characteristic in common. One domain directs DNA binding, while a second mediates formation of functional complexes with other proteins. For example, cooperative binding of the lambda repressor at adjacent operator sites is brought about by interacting carboxy-terminal domains²⁵, and this interaction is also a model for loop-forming contacts between proteins bound at more distant sites^{26,27}. The carboxy-terminal domain of TFIIIA is essential for correct transcriptional initiation, presumably because it binds to other components of the transcription complex.

There are hints of further structural classes for DNA-binding domains of sequence-specific transcription factors. The yeast GAL4 protein, which binds to upstream activating sequences, and the Simian virus 40 large-T antigen, which regulates transcription and stimulates DNA replication, may contain elements that are examples of such classes. The lesson of progress with TFIIIA is that structural characterization of such proteins and careful analysis of their binding sites can indeed make the apparent complexity of control sequences much less bewildering. □

1. Brown, D.B. in *The Harvey Lectures Ser. 76*, 27 (Academic, New York, 1982).
2. Miller, J., McLachlan, A.D. & Klug, A. *EMBO J.* **4**, 1609 (1985).
3. Rhodes, D. & Klug, A. *Cell* **46**, 123 (1986).
4. McCall, M. *et al.* *Nature* **322**, 661 (1986).
5. Engelke, D.R. *et al.* *Cell* **19**, 717 (1980).
6. Bogenhagen, D.F. *et al.* *Cell* **28**, 413 (1982).
7. Sakonju, S. *et al.* *Cell* **23**, 665 (1981).
8. Sakonju, S. & Brown, D.B. *Cell* **31**, 395 (1982).
9. Picard, B. & Wegnez, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 241 (1979).
10. Smith, D.R. *et al.* *Cell* **37**, 645 (1984).
11. Hanas, J.S. *et al.* *J. biol. Chem.* **258**, 14120 (1983).
12. Berg, J. *Science* **232**, 485 (1986).
13. Rosenber, V.B. *et al.* *Nature* **319**, 336 (1986).
14. Drew, H. & Travers, A. *Cell* **37**, 491 (1984).
15. Arnott, S. *et al.* *J. molec. Biol.* **81**, 107 (1973).
16. Tooze, J. *Molecular Biology of Tumor Viruses Part 2: DNA Tumor Viruses* 2nd edn (Cold Spring Harbor Laboratory, New York, 1981).
17. Dynan, W.S. & Tjian, R. *Cell* **32**, 669 (1983).
18. Rhodes, D. *EMBO J.* **4**, 3473 (1985).
19. Tso, J.Y. *et al.* *Nucleic Acids Res.* **14**, 2187 (1986).
20. Pabo, C. & Sauer, R. A. *Rev. Biochem.* **53**, 293 (1984).
21. Anderson, W.F. *et al.* *Nature* **290**, 754 (1981).
22. McKay, D.B. & Steitz, T.A. *Nature* **290**, 744 (1981).
23. Anderson, J.E. *et al.* *Nature* **316**, 596 (1985).
24. Hendrickson, W. & Schleif, R. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3129 (1985).
25. Johnson, A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 5061 (1979).
26. Dunn, T.M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 5017 (1984).
27. Hochschild, A. & Ptashne, M. *Cell* **44**, 681 (1986).

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The stability of biological nomenclature: yeasts

SIR—The strictures of Erzincioğlu and Unwin on the inconvenient instability of zoological nomenclature (*Nature* **320**, 687; 1986) apply equally to other kinds of organism, including the yeasts. For non-taxonomists, the rapidity with which yeast names change is farcical. For example, *Zygosaccharomyces fermentati*¹ was altered to *Saccharomyces cerevisiae* by Lodder and Kreger-van Rij in 1952², back to *Zygosaccharomyces fermentati* by Kudriavzev in 1954³, to *Saccharomyces montanus* by Phaff, Miller and Shifrine in 1956⁴, to *Torulaspora manchurica* in 1975 by van der Walt and Johannsen⁵ and finally (?) back again to *Zygosaccharomyces fermentati* by von Arx and his colleagues in 1977⁶. The same process continues even now. As part of a major work on yeast taxonomy⁷, C.P. Kurtzman gave descriptions of thirty species of the genus *Hansenula*, which he then proceeded to abolish in a paper published the same year⁸.

Part of the trouble comes from the romantic confusion of biological classification with evolutionary studies, exemplified by S. J. Gould's words⁹:

"taxonomies are not neutral hat-racks for the pristine facts of nature. They are theories that create and reflect the deep structure of science and human culture."

Too many taxonomists appear to subscribe to this kind of sentimental codswallop and seem quite unable to understand that instability of nomenclature seriously impairs the value of their work to biology as a whole. How many biologists who work with yeasts know that publications on *Saccharomyces carlsbergensis*, *Saccharomyces uvarum*, *Saccharomyces cerevisiae* or *Saccharomyces logos* might refer to the same yeast? At best, this is an inconvenience; at worst, it retards understanding of the biology of that yeast.

It would be perfectly practicable for there to be an international body, responsible for the nomenclature of each group of organisms, and briefed to give their names helpful stability.

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1. Naganishi, H. *J. Fermentation Stud.* **6**, 1-12 (1928).
2. Lodder, J. & Kreger-van Rij, N.J.W. *The Yeasts: a Taxonomic Study* (North-Holland, Amsterdam, 1952).
3. Kudriavzev, V.I. *Sistematika Drozhdzhei* (Akademii Nauk, Moscow, 1954).
4. Phaff, H.J., Miller, M.W. & Shifrine, M. *Antonie van Leeuwenhoek* **22**, 145-161 (1956).
5. van der Walt, J.P. & Johannsen, E. *S. Afr. Council Sci. Ind. Res. Res. Rep. No. 325* (1975).
6. von Arx, J.A., Rodrigues de Miranda, L., Smith, M.T. & Yarrow, D. *The Genera of Yeasts and the Yeast-like Fungi* (Centraalbureau voor Schimmelcultures, Baarn, 1977).
7. Kurtzman, C.P. in *The Yeasts: a Taxonomic Study* (ed. Kreger-van Rij, N.J.W.) 165-213 (Elsevier, Amsterdam, 1984).
8. Kurtzman, C.P. *Antonie van Leeuwenhoek* **50**, 209-217 (1984).
9. Gould, S.J. *Nature* **313**, 505-506 (1985).

Microtubule assembly in the axon

SIR—Bamburg *et al.* (*Nature* **321**, 788; 1986) present a novel view of microtubule assembly in the axon. They show that axonal elongation can be inhibited by an application of microtubule depolymerizing drugs or taxol at the growth cone. The same concentrations of these drugs applied to the neurite or cell body have no effect on motility. The authors conclude that the drugs may act by inhibiting microtubule assembly at the distal tip of the axon. They also postulate that unassembled tubulin is transported along the axon to the growth cone, and there adds on to existing microtubules.

As presented, the experimental measurements of axonal length with time are interpreted as a reflection of the extent of microtubule assembly. Such measurements of extent cannot be used to distinguish between effects of the drugs on the rate constants for assembly or disassembly. The microtubule-depolymerizing drugs could act by increasing the rate of disassembly at the tip. For this alternative explanation to hold, one must postulate only that microtubules at the growth cone, but not along the neurite, are in dynamic equilibrium with a pool of monomer. There is no need to require that a net assembly reaction occurs in this region. The drugs could have their effect by shifting the dynamic equilibrium towards monomer. The existence of such an equilibrium state is a feature of other models of microtubule control in axons. For example, if microtubules are transported in the assembled state, they must be disassembled at the end of the axon. Should such an equilibrium exist, sufficiently high concentrations of drug not only might inhibit neurite extension but also cause net retraction. That outcome is reported by Bamberg *et al.* Thus, these data do not demonstrate microtubule assembly at the ends of axons. More direct experiments are required to demonstrate this reaction.

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Disease gene relationship seen

SIR—A recent article by Royer-Pokora *et al.*¹ described the isolation of a gene that is abnormal in the X-linked form of the phagocytic disorder chronic granulomatous disease (X-CGD), which is thought to be due to a lesion in the NADPH-oxidase system of these cells². An unusual *b*-type cytochrome³ and a flavoprotein⁴ have been proposed as candidates for the polypeptide encoded by the Z-CGD locus, but the nucleotide

sequence of the cDNA clones isolated by Royer-Pokora *et al.* predicted a polypeptide that showed no significant homology to known sequences in the GENBANK or protein databases. The authors could not identify any potential haem-binding region in the primary structure of the X-CGD protein.

We have, however, found a region of the predicted X-CGD sequence that is similar to the haem-binding region of the cytochrome P-450 proteins, and in particular to cytochrome P-450 form PBc1 from rabbit liver⁵. The figure below illustrates the point, and identifies with an asterisk the cysteine thiolate ligand to the haem prosthetic group of the cytochrome.

X-CGD protein	L C G P E A L A E T
Cytochrome P-450	
form PBc1	V C* V G E A L A R M

The haem-binding region is highly conserved among cytochromes P-450, some of which have only a slight overall sequence similarity to one another. It is of interest that the two regions are located at equivalent positions in X-CGD and cytochrome P-450 (at about amino acid 435).

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1. Royer-Pokora, B. *et al. Nature* **322**, 32-38 (1986).
2. Tauber, A.I., Borregaard, N., Simons, E. & Wright, J. *Medicine* **62**, 286-309 (1983).
3. Segal, A.W. *et al. New Engl. J. Med.* **308**, 245-251 (1983).
4. Markert, M., Glass, G.A., & Barbior, B.M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3144-3148 (1985).
5. Leighton, J.K., DeBrunner-Vossbrink, B.A. & Kemper, B. *Biochemistry* **23**, 203-210 (1984).

Mankind's genetic bottleneck

SIR—Your correspondents^{1,2} question whether our interpretation³ of a small bottleneck in human evolution based on the patterns of distribution of β -globin haplotypes⁴ is correct and, if so, whether it is relevant to the origins of mankind. Our brief answer is that, although the modern distribution of these genes can in itself tell us rather little about where and how modern *Homo sapiens* originated, it is nevertheless the case that — given the assumptions of our very simple model and applying it only to β -globins — passage through such a bottleneck is an inevitable implication of the present-day distribution of β -globin variants. We attempted to 'root' our evolutionary tree by reference to recent work on human fossils, which suggests that the most ancient *H. sapiens* are found in Africa. It is of course the case

that the geography of β -globin variants is in itself compatible with an African, an Asian or a dual origin of mankind, but the fossil record does seem to indicate an African birthplace for modern humans. As geneticists fall among palaeontologists, we are not qualified to comment on the controversy as to the dating of African fossils, but have accepted the consensus view and used this (and not the β -globin tree) to suggest an African origin for mankind. Our deduction of a bottleneck at the origin of the rest of the world's human population is the simplest model consistent with the fossil data and the geography of the β -globins. Simplicity has forced us to make a number of assumptions.

One of these is that β -globin variants are selectively neutral. Nothing would delight us more than to learn that the possession of alternative haplotypes altered their carriers' ability to escape from sabre-toothed tigers, but in the absence of evidence to the contrary we have accepted the view, implicit in most theories of molecular evolution, that natural selection does not act on noncoding sequences of DNA. As both your correspondents point out, this is not necessarily correct; and there certainly exist quite feasible modes of selection on noncoding variants by virtue of their linkage to strongly selected alleles such as that for sickle cell haemoglobin⁷. As there is simply no information on this possibility, we have, with Occam, chosen the more straightforward alternative. The model also includes the arbitrary, but simple, suggestion that the four commonest modern haplotypes were at equal frequencies in the ancestral African population. This assumption is not central to the existence of a bottleneck, but does influence its size. The frequencies in modern African populations pointed out by Giles and Ambrose¹ could either reflect a conservation of their ancient values — suggesting an even smaller bottleneck in the emergent population — or a change within Africa arising from genetic drift in small populations (which could be many times larger than our supposed bottleneck of emigrants).

Despite the widespread popularity among palaeontologists of models of a multiple origin of mankind (for example ref. 7 of Van Valen), theoretical population genetics suggests that any population's ability to undergo speciation is so limited that it is extremely unlikely that *H. sapiens* arose simultaneously in different parts of the world from different ancestors. For example, using a simple model of change by genetic drift, Sewall Wright showed in the 1940s⁸ that the probability of a chromosome rearrangement with a selective disadvantage to hybrids of 5% becoming established in a local population of 200 individuals is about 10^{-5} per generation. The chance of this happening simultaneously in two separate populations is

about 10^{-10} per generation, which is extremely small, but may still be greater than the chance of simultaneous speciation in two separate populations. Van Valen's alternative suggestion — that of simultaneous global appearance of *H. sapiens* — implies extensive gene flow among its evolving populations, so extensive that both the ancestral *H. erectus* and the derived *H. sapiens* must be thought of as effectively sympatric. Sympatric speciation is itself difficult to accommodate into population genetics theory, and in Van Valen's scheme one is forced to explain the observed patterns of β -globin divergence by postulating long periods when large African and Eurasian populations of mankind were isolated from each other.

Our model, like many others, exists simply to show the evolutionary implications of an observed genetic structure. Its main point is to show that some of the demographic consequences of human gene frequency change are quite startling; startling enough, perhaps, to lead us to try to formulate a better model. A successful model would also, as we point out, have to explain why different genes seem to imply different demographic patterns during the spread of mankind.

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1. Giles, E. & Ambrose, S.H. *Nature* **322**, 21–22 (1986).
2. Van Valen, L. *Nature* **322**, 412 (1986).
3. Jones, J.S. & Rouhani, S. *Nature* **319**, 449–486 (1986).
4. Wainscoat, J.S. *et al.* *Nature* **319**, 491–493 (1986).
5. Antonarakis, S.E., *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 137–141 (1982).
6. Wright, S. *Genetics* **28**, 114 (1943).

Transfer of radioiodide to milk and its inhibition

SIR—Following the increased atmospheric levels of radioactivity resulting from the Chernobyl reactor accident¹, one of the main radiological concerns has been the entry of radioiodide into the human food chain through animal milk, and its subsequent uptake into the thyroid gland, as reported recently in your columns².

Current studies of transport mechanisms for various radionuclides in the mammary gland emphasize the rapidity with which blood-borne radioiodide can enter milk. The mammary gland of two goats was continuously infused for 15 min with ^{125}I (2.78 MBq; sodium iodide, AERE Harwell) through an indwelling polyvinyl chloride catheter placed in the external pudic (mammary) artery. Milk was completely removed by hand at 15 min intervals with oxytocin treatment (100 mUnits intravenously) and total radioactivity measured in a LKB Wallac 80000 gamma-sample counter. The total ^{125}I activity secreted into the milk rose rapidly to a peak concentration just 30 min after the start of infusion followed by a slower

decrease. The fractional activity of ^{125}I secreted in milk during a 2 h period was 1.0 and 1.5% of the infused activities.

The iodide concentrating activity of the mammary gland has long been recognized^{3,4}, and earlier work showed that the secretion of radioiodide into milk could be inhibited by competing anions such as perchlorate and thiocyanate^{3,5}. We repeated the above experiment by infusing sodium perchlorate (100 mg) close-arterially for 30 min before and during ^{125}I infusion. The total fractional ^{125}I activity transferred into milk during 2 h was reduced by 60–66%.

Perchlorate has previously been found to be a more efficient inhibitor of radioiodide transfer into milk than thiocyanate, iodide or iodate³. Thiocyanate was not concentrated in rabbit milk³, but we are unaware of any data that quantifies perchlorate transfer into milk. This information is urgently needed before perchlorate administration could be considered as a means of protecting against the entry of radioiodide into the human food chain⁶ or into breast-fed infants after a reactor accident⁷. The use of perchlorate is not unprecedented since the compound has been used without deleterious effects in nuclear medicine to block, for example, the uptake of radionuclides into the maternal thyroid during diagnostic investigations⁸. There is also a clear need to identify inhibitors which block mammary transport of other radionuclides, including fission products.

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1. Fry, F.A., Clarke, R.H. & O'Riordan, M.C. *Nature* **321**, 193–195 (1986).
2. Hill, C.R., Adam, L., Anderson, W., Ott, R.J. & Sowby, F.D. *Nature* **321**, 655–656 (1986).
3. Brown-Grant, K. *J. Physiol., Lond.* **135**, 644–654 (1957).
4. Brown-Grant, K. *Physiol. Rev.* **41**, 189–213 (1961).
5. Lengemann, F.W. *J. Dairy Sci.* **56**, 753–756 (1973).
6. Hoffman, F.O. *Health Phys.* **35**, 413–416 (1978).
7. Lechner, W., Huter, O., Daxenbichler, G. & Marth, C. *Lancet* **i**, 1326 (1986).
8. Coakley, A.J. & Mountford, P.J. *Br. Med. J.* **291**, 159–160 (1985).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*, but those that do should not be highly technical comments on Articles or Letters (where the Matters Arising section remains appropriate). □

Our daily lead

Josef Eisinger

The Lead Debate: The Environment, Toxicology and Child Health. Edited by R. Lansdowne and W. Yule. *Croom Helm*: 1986. Pp.276. Hbk £25, Pbk £14.95.

LEAD may be, with the exception of smoke, the oldest man-made environmental toxin. The manifold benefits which the element has bestowed on civilization — from plumbing to pigments — have been paid for heavily in human lives. Lead mining and manufacturing have of course been known as dangerous occupations since ancient times, but it was not until 1656 that Samuel Stockhausen, the first genuine occupational physician, presented clear evidence that lead was responsible for the particular ills and short life span of men and women engaged in these occupations.

Lead disease was, however, by no means confined to these and other lead workers. Lead was used as a wine additive for many centuries (it is a bactericide and has a sweet taste), and inadequately fired lead-glazed cooking utensils in 18th-century Germany, were just two of many causes of widespread non-occupational plumbism. In contrast, massive chronic exposures are, in our own times, rare, but ingestion and inhalation of low levels of lead in the environment has become inescapable for all. As a result, the body burden of lead in modern man exceeds that of prehistoric man by orders of magnitude. The lead-sensitivity of different human enzyme systems varies greatly, however, so that the health effects of our lead environment are subtle, variable and often escape correct diagnosis. It is therefore not surprising that the toxicity of prevalent lead exposures presents a difficult problem and often provokes impassioned debates.

The Lead Debate is a multi-authored compendium of articles centered around the health effects of non-occupational lead exposure, particularly in children. It attempts to distil a vast amount of relevant information into a small volume, in order to make it accessible to a broad range of readers, including medical and social workers, environmental scientists and policy makers. The attempt is, except for some lapses noted below, successful.

The book is divided into three parts, of which the first deals adequately with the historical, chemical, geological and medical background of lead. Environmental lead disease is of course best controlled by limiting the amount of lead which industrial societies inject into air, water and

soil; and legislation which controls the amount of alkyl lead added to petrol represents a step in the right direction. The next line of defence against chronic exposure is clearly an effective screening programme for populations at risk, particularly for children, and the book includes a, regrettably, brief section on the methodology of measuring levels of lead in humans and in the environment.

The two screening methods which have been used more than any other are based on the concentration, in blood, of lead (PbB) and of zinc protoporphyrin (ZPP), respectively. The latter is a lead-induced, fluorescent metabolite which mimics the haem in haemoglobin during erythropoiesis and is most conveniently measured

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Collecting dust particles from a school in London to test for lead levels.

by a dedicated, portable instrument called a haematofluorometer. Of these two assays, the ZPP assay is much cheaper, requires much less blood (generally a drop obtained by finger stick), provides an immediate result, and has a significantly higher correlation with the symptoms of chronic lead disease than PbB assays. For these reasons, the US Centers for Disease Control has recommended ZPP assays for the primary screening of children for lead poisoning and, incidentally, for iron deficiency anaemia. According to the Public Health Foundation, more than 700,000 such screening tests are performed by state health departments in the US each year, over 90 per cent of which use the ZPP haematofluorometer.

In light of this, it is strange that the chapter devoted to lead exposure measurements does not mention this instrument! It is even stranger that, despite its admitted advantages, the ZPP assay is "not recommended at blood lead levels of about 15 µg/dl". The reason given is that there is only a weak correlation between ZPP and PbB below that level, which is

well within the range considered "normal" (≤ 25 µg/dl). Leaving aside the questionable relevance of this correlation, it is not clear why an efficient screening tool like the ZPP haematofluorometer should not be used to identify children with PbB levels above 15 µg/dl, for they have, without doubt, the greatest need for medical attention!

The second part of the book offers several well organized and informative chapters on the distribution and sources of lead exposure. Because they are written by only two authors, they avoid the unevenness of the earlier sections (where the references to primary sources are occasionally too sparse). Here one finds an account of, possibly, the most significant and elegant environmental lead study, in which the variation in the isotopic ratio (Pb^{206}/Pb^{207}) of the alkyl lead additives used in petrol was varied slowly over a period of six years. This variation was found to be closely linked to the isotopic ratio of airborne particulate lead as well as by the isotopic ratio of lead in the blood of subjects living in Turin and the surrounding countryside, where the experiment was conducted with the cooperation of Italian oil companies.

This and similar studies in the US and UK have established that anthropogenic lead — principally from petrol combustion — is the most important component of lead pollution. Once it is injected into the air this lead finds its way into the human body, primarily by way of the food and water supply. Excessive concentrations in tap water have long haunted regions where lead pipes continue in use, as for instance in Scotland. Reductions in the lead content of water have now been achieved by raising the pH of the water and buffering it with the aid of sodium hydroxide and inorganic phosphate additives.

In the UK and the US, the most serious cases of lead poisoning in children can be traced to lead-pica — the chewing of either peeling paint or of painted articles, prompted by the sweet taste of the lead ion (Pb^{2+}). (The term pica is derived from the much maligned magpie, *Pica pica*.) The strategy for prevention is clear: removal of old paints and the use of non-lead paints for interior surfaces. What the book's authors fail to mention is that well-meaning government regulations are, because of their enormous cost, rarely enforced, so that effective screening programmes for young children again offer the best hope for finding and eliminating trouble spots and for providing needed medical attention.

The remainder of the *The Lead Debate* addresses the health effects of ingested lead, with particular emphasis on the

Photo Coo-op

psychological development of children exposed to ordinary environmental lead levels. It opens, appropriately enough, with a discussion of the methodological difficulties of interpreting the numerous studies which attempt to identify the disease-exposure relationship. It is unfortunate that most of these studies use PbB as the only exposure index, although the most important study in America does include dentine lead as a cumulative index. The warning against equating correlations with causal relationships are well taken, and the critical commentary on the conclusions drawn from studies exploring lead-effects on intelligence and behaviour are particularly helpful. Although caution in the interpretation of low-level exposure data is clearly in order, there is no lack of evidence, from epidemiological studies and animal experiments, that massive and moderate exposure produces measurable neurobehavioural effects.

The enduring dilemma surrounding the lead debate is that the threshold of lead-related disease continues to defy definition. This should surprise no one for it requires a quantitative determination of such multivariate parameters as intelligence, behaviour and learning. But supposing that such a threshold could be translated into an indicator of exposure (for example PbB or ZPP levels, *vide supra*) a second, even more difficult question faces policy makers: what is the fiscal value which society attaches to the elimination of marginal plumbism? In crasser terms, how much is the taxpayer willing to pay, in order to raise the average intelligence of the population by, say, 1 per cent?

It is unfortunate that these tricky issues are rarely touched upon in *The Lead Debate*. Usually, the authors prefer to remain on the safe technical level and to quote from general conclusions of governmental commission reports. A chapter on real and perceived risk of lead disease compared to other health insults which we are exposed to, together with estimates of their cost of abatement, would have been welcome (c.f. *New England Journal of Medicine* 1982:1392).

On the other hand, the contributors have collected a lot of extremely useful scientific information which will undoubtedly help to keep the continuing debate about plumbism on a firm scientific footing. They have succeeded in avoiding polemics, albeit at the cost of avoiding novel or controversial approaches. Nevertheless *The Lead Debate* is an important and useful interdisciplinary review of where we stand in our efforts to unravel the complexities of this ancient environmental hazard. □

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Visitors from the past

Theya Molleson

Lindow Man: The Body in the Bog. Edited by I.M. Stead, J.B. Bourke and Don Brothwell. *British Museum Publications, London:1986. Pp.208. £15.*

THE great interest that attaches to any bog body derives not simply from the rarity of such finds but also from the intrinsic nature of the body: it is almost a visitor from the past. Such bodies should tell us more of the life and character of the earlier people. To deduce the age and sex of a body is one thing; but to know something of his clothing and ornaments and to discover the ingredients of his last meal and, above all, to see his face is somehow to perceive the whole anima of his time. I don't think that we should be disappoint-

one of the most significant bog findings in Britain and the greatest challenge to archaeological conservation. Within days the police moved out, the hospital wanted the body removed and the conservationists were asked to take over. They were expected to prevent any deterioration of the body while allowing endless study, photography, sampling and dissection. And all they knew from previous experience was what *not* to do. By a conscientious and didactic approach it does seem that Lindow Man has been preserved for posterity.

The volume, *Lindow Man*, presents the progress reports of the wide range of exploratory studies — from identification of the surrounding peat and of the pollen, cereals and parasites in the stomach to interpretation of the context both geographic and cultural. Some papers are too clearly hurriedly written; others tend to be discursive for want of factual evidence. It is surprising how little appears to be known of our Celtic heritage.



Lindow Man has his back cleaned, taken from *The Bog Man and the Archaeology of People* (British Museum Publications, Pbk £5.95). In this book, Don Brothwell gives a briefer, less technical account of the Lindow project. Lindow Man is also on display at the British Museum in London, together with the results of the research programme, as part of the exhibition *Archaeology in Britain* which runs until 15 February 1987.

ted when our expectations are but inadequately realised. Lindow Man has been thoroughly studied but has yet to tell his full story and, not least, to declare his age.

Enormous credit must go to Rick Turner, the county archaeologist for Cheshire, where Lindow Man was discovered in 1984. He was able immediately to appreciate the archaeological potential that lay in just a fold of skin exposed in the section at the peat cutting. He even managed to convince all concerned that the correct procedure then was to delimit the area of the body, if body there was; to cut it out and crate it for removal to the local mortuary. At this stage the investigation was still a police matter, and not

Concepts of sacrificial ritual emanating from the bog finds in Europe have dominated the approach to the study of Lindow Man. Several workers have found it hard to view their data independently of these theories so that the final interpretation tends to be prejudiced. Yet the information as presented is open to other constructs than that of a ritual killing on the lines of the Danish Bog burials. And that is the advantage of the presentation of the independent studies in detail and in one volume. Readers can make up their own minds. □

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Dial a vector

Tim Harris

Cloning Vectors: A Laboratory Manual.

Edited by P.H. Pouwels, B.E. Enger-Valk and W.J. Brammar. *Elsevier:1985. Approximately 400 sheets in Loose-leaf binder. \$55, Dfl.160.*

LABORATORY manuals have become a popular means of disseminating the complicated protocols of modern molecular genetics, the best example being *Molecular Cloning — A Laboratory Manual*, referred to by some as the cloning bible, by Maniatis *et al.* Some of these manuals, like the above, combine theory with practice while others are simply sophisticated recipe books. Although described as a laboratory manual, *Cloning Vectors* is a directory of the vectors currently available for introducing genes into a variety of organisms. The vital statistics of some 300 vectors, both plasmid and phage in origin, are described by means of clear, single-page diagrams. About one-third of the plasmids are those used for the most commonly employed cloning host — the gram negative bacterium *E. coli*. The remaining chapters cover vectors for gram positive bacteria (for example, *Streptomyces*), fungi (including yeast) and plant and animal cells.

The work is comprehensive, as up to date as might be expected in a fast-moving field, and the clarity and simplicity of the figures is impressive. Provided you know what you are looking for, the salient facts are easy to obtain. It is possible to use the summary tables at the end of each chapter to locate vectors with desired characteristics (one of the objectives of the book) but it's a pity little attempt was made to rank or distinguish commonly used vectors from more esoteric and not necessarily more useful ones. The loose-leaf format of the directory is designed so that annual updates can be included; some of the newer cosmid vectors for genome walking and vectors for insect cell expression can be expected*.

The lack of theoretical back up — the introductions to each chapter are only one or two pages — indicates that this book is not intended for the individual graduate or postdoctoral molecular biologist. Rather, like a telephone directory, it is a reference book that every genetic engineering lab will want access to, be it on their library, laboratory or office shelf. □

*These are indeed included in the first update which will be available in September. Price \$20, Dfl. 60. Orders for the main manual plus three annual updates are entitled to the reduced price of \$97.75, Dfl. 287.

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History isn't bunk

John H. Lawton

Modeling Nature. Episodes in the History of Population Ecology. By Sharon E. Kingsland. *University of Chicago Press: 1985. Pp.267. \$27.50, £23.50.*

AS EVERY schoolboy knows, Henry Ford thought that history was bunk, a view that is certainly common, if not universally held by many creative and successful scientists about their own subject. There is, after all, too much to read about what is happening today, still less about events 50 or more years ago. But a sense of perspective is no bad thing in any walk of life, and science is no exception, as Sharon Kingsland's book makes plain.

The book is a detailed history of the rise of population ecology from its early beginnings in the writings of Frederic Clements and Stephen Forbes (who in turn drew their inspiration from Charles Darwin, Herbert Spencer and so on, but historians have to stop going backwards somewhere), through Alfred Lotka, Raymond Pearl and Vito Volterra, and from there on a direct intellectual route via Georgii Gause to Evelyn Hutchinson, David Lack

and Robert MacArthur. There were, of course, other contributors, some major some minor, but this pedigree is particularly clearly defined, and gives a good indication of the scope of the book, and the time-scale involved.

Reviewing books is often a chore, is usually educative, but is only infrequently a real pleasure. *Modeling Nature* unfolds like a good detective story, and is a delight to read for at least two reasons. It is a beautifully written, well-crafted account of a complex subject; although I thought I knew the roots to population ecology fairly well, I found that many of my ideas were either fuzzy or wrong. It is extremely useful to have the key intellectual advances so clearly laid out. Second, and probably more important, the book is about people as well as ideas. It was, after all, no lesser historian than Edward Gibbon who said that history is "little more than a register of the crimes, follies, and misfortunes of mankind"! One would hesitate to use quite these terms to describe the history of population biology, but the founding fathers were not modest angels. They were certainly clever, but they were also — in varying degrees — opinionated, scheming and paranoid, as well as insightful, helpful and wise. And all of them were men, which in itself is an interesting comment on the sociology of

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science, although Kingsland does not open this particular can of worms.

Population ecology is currently in the throes of a series of intense and, at times, acrimonious debates. The arguments have several overlapping strands, including the role of density-dependent processes in population regulation, the importance of interspecific competition in structuring communities, the value of mathematics in ecology, and the proper way of doing science that would solve all these problems if only everybody would adopt the true way. Not one of these problems is new. All have been debated with just as much ferocity in the past, and what history shows is that the entrenched positions of the principal assailants have almost always proved to be misguided, irrelevant or wrong. History isn't bunk; it gives a sense of proportion.

Confronted with the realisation that we have been here before, sometimes more than once, it is easy to believe that population ecology has simply wandered round in aggressive circles, getting nowhere since Lotka published *Elements of Physical Biology* in 1925 and Volterra his famous letter to *Nature* a year later. Nothing could be further from the truth. Population and community ecology have made great strides over the past 60 years, despite the complexities of natural communities. From the fires of strong disagreement ultimately emerges the phoenix of scientific progress. But a better sense of history might reduce the sparks currently flying round population ecology, and help to generate less heat, more light and greater tolerance. □

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Plants in distress

Richard Dixon

The Biochemistry and Physiology of Plant Disease. By Robert N. Goodman, Zoltán Király and K.R. Wood. *University of Missouri Press*: 1986. Pp. 433. \$45.

RESEARCH in plant pathology is now entering an exciting period. Pathologists are becoming increasingly aware of the potential of new molecular techniques, while plant biochemists and molecular biologists are showing corresponding interest in plant-pathogen interactions as important systems for studies of cellular recognition, metabolic integration and gene expression. Any new, comprehensive review of this field should be judged in terms of its success in presenting the bases (often multidisciplinary) of current concepts, and its exposition of new experimental approaches. My first impressions of *The Biochemistry and Physiology of Plant Disease* were of a magnum opus, among whose wealth of detail would surely be found the signposts to future research. A full reading of the book left me impressed by the scale of the authors' understanding, but somewhat disappointed by the book's lack of direction and its conclusions.

Most chapters consist of brief descriptions of the biochemistry or physiology of a particular aspect of the healthy plant (respiration, photosynthesis, cell walls and so on) followed by detailed analyses of the effects of infection under the separate headings of viruses, bacteria and fungi. Taken together, the introductory sections almost make up a basic plant bio-

chemistry text, good in some sections (particularly cell walls), but in others too condensed to be of much value to non-biochemists (for example, the glyoxylate cycle is presented with no mention of its role in metabolism). Although the conceptual aspects are presented critically and lucidly, there are a relatively large number of material inaccuracies, particularly in the introductory biochemical sections — tyrosine is not only found in the *Gramineae* (p.211), acetyl CoA units are certainly not condensed to yield malonyl CoA (p.212) and phenylalanine is not altered by hydrolases (p.226).

The organization of the material, based on physiological divisions rather than temporal sequences of events, has inevitably led to repetition of information under different headings, although this is less apparent in the sections on viruses. Such repetition in a long work is often no bad thing, but some important topics are never fully dealt with in one section and appear piecemeal throughout the book. The accounts of the *Agrobacterium* system, fungal and host elicitors, and induced host gene expression fall into this category.

The scope of the book is, of course, clear from its title, and the depth of treatment and excellence of the illustrations will no doubt ensure its success as a standard text for advanced students. I was, however, disappointed by the lack of attention to genetics, both classical and molecular (viral nucleic acid metabolism excluded), as it is from the application of recombinant DNA techniques that the most promising developments in plant pathology will probably arise. In this respect, I feel that the authors' honestly-confessed lack of agreement in several areas, particularly the molecular basis of disease resistance, rather robs the book of an important component — that of informed prediction, even if based on personal prejudice. The search for avirulence, virulence and resistance genes is now a central effort in plant pathology, and more speculation in these areas, at the expense of repetition of correlative phenomena reviewed earlier in the book, would have increased the impact of the final "rounding-off" chapter on disease resistance. Likewise, the concluding sections to the individual chapters, each headed "Comparative Analysis of Disease Physiology", simply summarize rather than integrate the views of the individual authors.

Such problems apart, it is perhaps unfair to criticize the book too much for what it does not cover. It stands as a detailed source of information on physiological plant pathology, one which will certainly be widely read. □

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Baking and brewing in Egypt around 2400 BC, taken from the Open University study pack on Biotechnology (PS621, £119). Designed for those with no specialist knowledge, the pack consists of over 600 pages of text divided between eight books, including two volumes Laboratory to Marketplace which look at the impact of biotechnology on a number of key industries, a series of case studies and two books of supporting technical material. Although the modular pack is designed to be free-standing, there is an associated set of six video tapes (£115 each or £299 for the set). Available from Learning Materials Sales Office, The Open University, PO Box 188, Milton Keynes MK7 6DH, UK.

Aleutian terranes from Nd isotopes

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Nd isotope ratios substantiate the identification of oceanic crustal terranes within the continental crustal basement of the Aleutian island arc. The oceanic terranes are exposed in the westernmost Aleutians, but to the east, they are completely buried by isotopically distinct arc-volcanic rocks. Analogous oceanic terranes may be important components of the terrane collages that comprise the continents.

CONTINENTAL crust is a mosaic of structurally coherent terranes. Palaeomagnetic investigations have revealed that the terranes often originated within ocean basins, or at their margins, and travelled great distances before 'docking' at continental margins. The crust of the Aleutian island arc is analogous to that of many of these far-travelled terranes, and yet is simpler in structure, having not yet experienced the deformation and metamorphism which accompany terrane suturing. The Aleutian crustal building blocks are terranes themselves, and include oceanic crust, back-arc crust and subduction-related arc magmas. We show here that Nd isotope ratios are remarkably successful in identifying Aleutian terranes. We then define the lateral and vertical variations in Aleutian crustal architecture, and contrast a cross-section of oceanic (back-arc)-dominated crust in the western Aleutians with our previously documented¹ arc-magma-dominated crust in the eastern Aleutians.

Recent volcanic rocks

As a starting point, we note the isotopic homogeneity of Nd ($\epsilon_{\text{Nd}} = 7.30 \pm 1.25$; see Table 1 for definition) in a wide compositional spectrum of recent Aleutian volcanic rocks (see Figs 1, 2). This isotopic monotony contrasts with the distinctive chemical and mineralogical trends developed during the magmatic evolution of the volcanic rocks^{2,3}. Although there is no agreement as to how the Nd isotopic homogeneity was acquired⁴⁻⁸, for the present purpose it is more important to establish that the narrow range of ϵ_{Nd} that characterizes magmatically evolved Aleutian lavas was not acquired in the crust. To establish this we show that the same narrow range extends to minerals that have fractionated at depth from primitive (mantle-derived) magmas. For example, voluminous pyroclastic flows from Moffett volcano, Adak island, central Aleutians (Fig. 1), contain cumulate-textured xenoliths of mafic and ultramafic composition⁹. The xenoliths are mid-crustal crystalline wall rocks of dykes that carried the magmas to the surface. The xenoliths represent magma compositions ranging from olivine tholeiite (capable of precipitating Ni-rich olivine, Cr-rich clinopyroxene and Cr-rich amphibole, the cumulate minerals of xenolith MM76-102)⁹ to andesite (capable of precipitating clinopyroxene, amphibole and plagioclase, the cumulate minerals of the least magnesian xenolith, MM76-10)⁹. Nd isotope ratios of three of these xenoliths and the host andesite are the same (Table 1); thus ϵ_{Nd} is independent of crustal-level fractionation processes, and is characteristic of mantle-derived arc magmas. ¹⁰Be, a cosmogenic nuclide (half-life 1.5 Myr) which is present in young volcanic rocks of the arc, is also present in the most mafic of the xenoliths, MM76-102¹⁰ (the only Moffett xenolith analysed so far). From the standpoint of tectonics, an Aleutian cross-section would include these volcanic and plutonic rocks in the same terrane.

As a second example, ultramafic (olivine-clinopyroxene) xenoliths from Moffett's sister volcano Adagadak (see Fig. 1) are

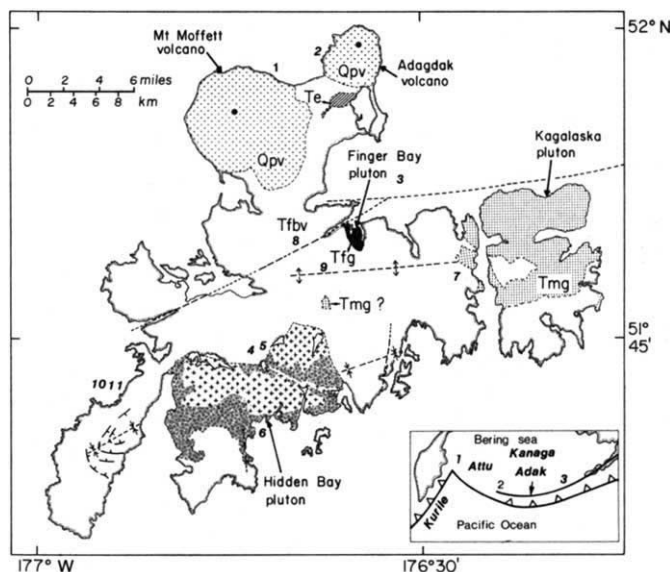


Fig. 1 Generalized geological map of Adak island⁴⁰, in the Central Aleutian island arc. For the Hidden Bay pluton, distribution of more silicic (>60% SiO₂, 'granodiorite') interior and less silicic (<60% SiO₂, 'diorite') exterior is after ref. 41. The plutons intrude the Finger Bay Volcanics (areas with no symbol on the map). Adak xenolith localities are Mt Moffett (51°58.01' N, 176°43.55' W, locality 1) and Mt Adagadak (51°58.78' N, 176°37.36' W, locality 2). These and numbers 3-11 refer to the location of samples listed in Table 1. The generalized inset map shows the locations of Attu, Kamagata and Adak islands and of the Komandorski back-arc basin (1), Amchitka Island (2) and Unalaska Island (3). Tooth marks indicate the trench segments that have associated active volcanoes.

ductilely deformed tectonites^{9,11}. The ϵ_{Nd} values of one of these tectonites is +7.2 (Table 1), and it contains small amounts of ¹⁰Be (ref. 10). Thus, we infer that the tectonites are geochemically related to the magmas of the recent volcanoes. On a tectonic cross-section, we infer that the deformation (which by textural arguments occurred at high temperature) is recent, and thus represents an active tectonic feature of the arc. We have proposed^{1,11,12} that the rocks are early-stage cumulates of arc magmas that reside in the sub-arc asthenosphere, which extends to depths as shallow as the Moho under the present arc. Stated in tectonic language, the brittle-ductile transition has reached shallow (Moho) levels directly under the Aleutian arc (see Fig. 3a). Note that the ϵ_{Nd} values for recent Aleutian volcanic rocks are higher than those for many other arcs with older continental basement, and apparently these high values extend to the Kurile Arc, based on two analyses (Table 1) which are within the narrow range of the Aleutian values.

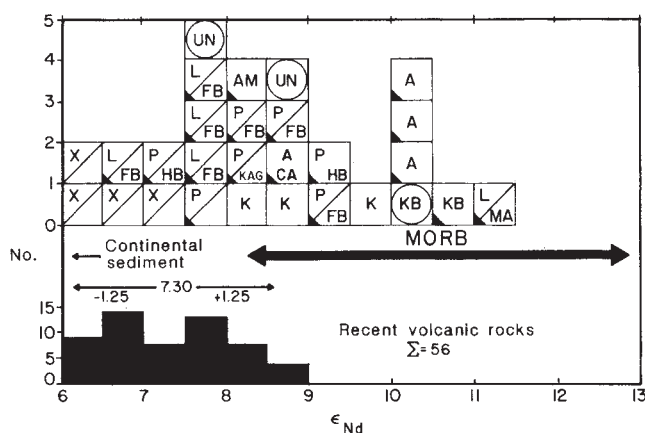


Fig. 2 Histogram of ϵ_{Nd} values of Aleutian volcanic and plutonic rocks^{18,24}, plotted relative to a BCR-1 ϵ_{Nd} value of -0.4 (ref. 7). $\square$, Adak: L, lava (FB, Finger Bay; MA, magnesian andesite); X, xenolith; P, pluton (KAG, Kagalaska; HB, Hidden Bay; FB, Finger Bay). $\square$, Kanaga xenoliths; $\square$, Amchitka pluton; $\square$, Unalaska pluton; $\square$, Attu (CA, calc-alkaline); $\square$, Kamchatka basin. $\square$, LDGO; $\square$, literature. Recent volcanic rocks^{6-8,14,15,24,42,43}, mid-ocean-ridge basalts (MORB)^{30,31}, Unalaska pluton (Captains Bay, ref. 24) and Komandorski basin basalt⁷ are all from the literature. (Other analyses, including an independent analysis of the Komandorski basin basalt, are listed in Table 1.) Note the coincidence of: (1) Attu island lavas and Komandorski basin basalt; (2) Finger Bay lavas (early Tertiary) and 56 Recent volcanic rocks; (3) Adak xenoliths (from Moffett and Adagdak volcanoes) and Recent volcanic rocks. Note that some Kanaga xenoliths and Tertiary plutonic rocks plot between recent volcanic rocks and the Attu-Komandorski basin lavas. We interpret the rocks with intermediate ϵ_{Nd} as geochemical hybrids.

A singular lava

It has been known for some time that lavas of the Aleutian arc have variable Sr and Pb isotopic compositions^{13,14}. The most isotopically extreme lava comes from a thick, magnesian andesite flow or sill at the base of Mt Moffett (Fig. 1). It has very non-radiogenic Sr and Pb (ref. 14) and very radiogenic Nd ($\epsilon_{\text{Nd}} = +11.3$ (ref. 15; see Table 1), a value which is at the high end of the mid-ocean-ridge basalt (MORB) range (Fig. 2). This lava also differs in its trace element content from all other Aleutian lavas analysed so far¹⁴. We infer that a region under the main volcanic arc, but at unknown depth, has MORB-like character. Some possibilities are: the subducted oceanic crust (but without isotopic or trace element contamination from hydrothermal alteration by sea water), the overlying asthenosphere, or even, as we will see, the crustal or mantle lithosphere.

Older magmatic rocks

The magmatic history of the Aleutian arc reaches back to the early Tertiary¹⁶⁻¹⁹. On Adak island, the early volcanic and volcanoclastic rock units, mapped as the Finger Bay Volcanics (Fig. 1), are cut by plutons of several ages. On the westernmost Aleutian Islands (Attu and the other Near Islands) and the Komandorski Islands there are no active volcanoes (plate motion up to ~ 5 Myr ago was nearly strike-slip)²⁰, but there are excellent exposed sections of Tertiary volcanic rocks, cut by intrusives^{18,21}. Isotopic contrasts between the Tertiary lavas of Adak and Attu¹⁸ constitute our first indication that the Aleutian arc crust is not comprised entirely of arc magmas of the type that are erupting today. The early Tertiary ($^{40}\text{Ar}/^{39}\text{Ar}$ crystallization ages > 50 Myr¹⁶) Finger Bay volcanics from Adak have the same ϵ_{Nd} as recent Aleutian volcanic rocks (see Fig. 2). In contrast, the earliest volcanic rocks on Attu (probably 20–30 Myr in age)¹⁸ have higher ϵ_{Nd} (well within the range of mid-ocean-ridge or back-arc-basin basalt; see Fig. 2) than any magmatic rocks of any age in the arc (except, as always, the

Mg-andesite from Adak). However, not all Attu lavas have high ϵ_{Nd} . A hornblende andesite representing the volumetrically minor, youngest (Miocene) volcanic rocks on Attu has ϵ_{Nd} that is in the range (within error) of values of recent volcanic rocks. These data from Attu can be explained either by evolution of magmas from high to low ϵ_{Nd} during the Tertiary, or by an origin of the earliest Attu magmas in a back-arc setting. A third alternative, origin at a mid-ocean ridge, is not consistent with recent syntheses of the plate kinematics of the North Pacific^{22,23}. The apparent invariance of ϵ_{Nd} with age over the same time period (30 Myr) on Adak and Unalaska^{18,24} (an eastern Aleutian island; see Fig. 1) argues against isotopic evolution of the arc from high to low ϵ_{Nd} (ref. 7). The similarity of the oldest Attu lavas and a Miocene back-arc lava from the Komandorski basin (Table 1), not only in ϵ_{Nd} but in Sr isotope ratio⁷ and a wide range of lithophile elements (in particular, the rare earths)¹⁸ supports our proposal that the Attu section represents lavas and interbedded cherts and turbidites from a back-arc basin analogous to the younger Komandorski basin. Subsequently, these 'basement' rocks were intruded by subduction-related dykes, and in places they are overlain by basaltic and andesitic lavas fed by the dykes, but this arc-related magmatism is minor in volume.

Some units of plutons that intrude the Finger Bay Volcanics on Adak (see Fig. 1) have ϵ_{Nd} values which are slightly higher than those of the highest- ϵ_{Nd} volcanic rocks (see Fig. 2). Within both the Hidden Bay and Finger Bay pluton it is the most mafic samples (the gabbros) that have these high ϵ_{Nd} values. McCulloch and Perfit's²⁴ analyses of an Unalaska pluton show the same trend. The variability within the plutons indicates a heterogeneous source, or mixing of igneous rocks from more than one source. It is somewhat perplexing that the most mafic lavas do not show this tendency towards high ϵ_{Nd} . We believe that this is the first indication that the volcanic and plutonic rocks are petrogenetically distinct. We tentatively identify the high- ϵ_{Nd} end-member as oceanic crust buried within the arc crust.

Crustal and mantle tectonites

A remote cluster of later Tertiary basaltic intrusives on the magmatically inactive southern part of Kanaga Island (~ 30 km west of Adak) has yielded mafic and ultramafic xenoliths which contrast strongly with those from Adak (discussed above). To the previously documented mineralogical contrasts²⁵⁻²⁹, most obviously the fact that they are amphibole-free, unlike their Adak counterparts, we now add a contrast in ϵ_{Nd} . Both mafic and ultramafic tectonites have higher ϵ_{Nd} than the Adak cumulate and tectonite xenoliths. The highest value ($\epsilon_{\text{Nd}} = +9.6$) lies well outside the range of Aleutian lavas, and within the range of both mid-ocean-ridge and back-arc-basin basalts^{30,31}. But the xenoliths are clearly not pieces of the subducted oceanic plate (from 100 km depth), for their mineralogy indicates equilibration at much shallower depths, within the spinel ilmenite stability field or shallower (for the plagioclase-bearing but garnet-free mafic granulites). We have proposed¹ that the xenoliths originated as buried pieces of oceanic or back-arc lithosphere. They have been almost completely recrystallized and therefore retain few textural or mineralogical clues to their origin. They also appear to be hybrids: they are geochemically intermediate between arc and MORB types. Assimilation of some of this hybridized lithosphere by arc magmas may account for the unusually high ϵ_{Nd} of some of the plutons, noted above.

By analogy, this interpretation finds some support in a study of ophiolites. In the Troodos ophiolite³¹, a range of ϵ_{Nd} values can be interpreted as reflecting two-component mixing (although at a depth greater than that envisioned here) between a MORB-like ($\epsilon_{\text{Nd}} \geq +8$) and a continental-crustal (ϵ_{Nd} negative) source. The Sr isotopic composition of the Troodos ophiolite rocks varies widely ($^{87}\text{Sr}/^{86}\text{Sr}$ ranges from 0.70415 to 0.70705), and is independent of the Nd isotopic values. Much the same can be

Table 1 Isotopic results from the Aleutian arc

Rock type	Locality* (ref.)	$^{143}\text{Nd}/^{144}\text{Nd}$ (Laboratory) [†]	$\epsilon_{\text{Nd}}^{\ddagger}$	Nd (p.p.m.)	Sm (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}^{\dagger}$	Rb (p.p.m.)	Sr (p.p.m.)
Cumulate xenoliths, Moffett volcano,								
Adak								
MM76-102 olivine-clinopyroxene	1 (9)	0.512170 (14) (C)	6.31 ± 0.28	8.5	2.0	0.703119 (25)	4.91	246
MM-DK olivine-hornblende gabbro	1 (9)	0.512198 (21) (C)	6.86 ± 0.42	3.6	1.1	0.703270 (33)	3.20	469
MM76-10 hornblende gabbro	1 (9)	0.512165 (18) (C)	6.22 ± 0.35	8.36	1.95	0.703309 (33)	5.53	426
ADK54 host lava	1 (9)	0.512984 (17) (L)	6.20 ± 0.34	8.23	2.66	0.702998 (42)		745
Tectonized Xenoliths, Adagdak volcano,								
Adak								
ADAG81-DR clinopyroxenite	2 (11)	0.512215 (16) (C)	7.19 ± 0.31	1.33	0.64	0.703355 (63)	0.103	31.3
Arc lavas								
B11/538 (Chirinkotan volcano)	— (§)	0.512218 (15) (C)	7.24 ± 0.30	19.2	4.14	0.703130 (38)	34.7	596
Kurile basalt								
116/81 (Bogdan Khmel'nitskii Volcano, Itirup)	— (§)	0.512243 (18) (C)	7.74 ± 0.35	23.0	6.8	0.70287	34.3	518
Kurile basalt								
ADK 53 Aleutian andesite	1 (14, 15)	0.513244 (22) (L)	11.3 ± 0.43	33.0	5.7	0.702795 (31)	16.4	1,780
Komandorski basin								
DSDP191 basalt	Inset (38)	0.513210 (22) (L)	10.6 ± 0.44	14.1	4.65	0.70289 (3)	7.3	173
Arc plutons								
FB53 gabbro	3 (39)	0.513144 (19) (L)	9.3 ± 0.38	13	3.1	0.70303 (3)	18	728
FB97 gabbro	3 (39)	0.513095 (19) (L)	8.4 ± 0.38	17	3.8	0.70326 (7)	25	753
FB61 monzodiorite	3 (39)	0.513125 (21) (L)	8.9 ± 0.42	38	7.7	0.70336 (4)	58	514
HB7-10 gabbro	4 (41)	0.513152 (20) (L)	9.4 ± 0.40	12.8	3.51	0.70305 (5)	28	735
HB7-16 diorite	5 (41)	0.513062 (22) (L)	7.7 ± 0.44	12.2	2.94	0.70323 (4)	19	1,140
HB5-137 granodiorite	6 (41)	0.513032 (22) (L)	7.1 ± 0.44	—	—	0.70328 (4)	23	601
K7-32A granodiorite	7 (41)	0.513089 (21) (L)	8.3 ± 0.42	10.7	2.36	0.70350 (4)	41	730
AM33 granodiorite, Amchitka	— (18)	0.513098 (23) (L)	8.4 ± 0.46	14.9	3.64	0.70330 (3)	26	400
Arc Lavas								
Tertiary, Adak								
LB8-10A basalt	8 (18)	0.513015 (25) (L)	6.8 ± 0.50	9.8	2.79	0.70332 (3)	9.7	523
LB80-39 basalt	9 (18)	0.513053 (17) (L)	7.6 ± 0.52	35.1	8.35	0.70316 (3)	53	526
BW8-R30 basalt	10 (18)	0.513061 (27) (L)	7.7 ± 0.54	27.2	6.29	0.70296 (3)	15.4	1,010
BW8-R36 basalt	11 (18)	0.513052 (17) (L)	7.6 ± 0.34	14.6	3.81	0.70306 (4)	14.3	900
Tertiary, Attu								
SB80-1A basalt	— (18)	0.513199 (18) (L)	10.4 ± 0.36	10.1	3.22	0.70357 (3)	4.9	183
H09-25B basalt	— (18)	0.513203 (17) (L)	10.5 ± 0.34	6.6	2.25	0.70369 (3)	11	313
H09-14B basalt	— (18)	0.513186 (22) (L)	10.1 ± 0.44	13.4	3.75	0.70281 (4)	4.3	229
AT80-32 andesite	— (18)	0.513114 (23) (L)	8.7 ± 0.46	11.1	2.70	0.70306 (4)	11	468
Tectonized xenoliths, Kanaga Island								
KAN80-6-5 peridotite	— (27)	0.512339 (19) (C)	9.62 ± 0.37	1.42	0.69	0.706427 (38)	0.74	39.0
KAN80-6-69 wehrlite	— (26)	0.512272 (23) (C)	8.31 ± 0.45	3.09	0.972	0.703843 (40)	0.318	30.6
KAN80-6-27 2-pyroxene granulite	— (28)	0.512286 (23) (C)	8.58 ± 0.44	1.89	0.70	0.703329 (50)	0.27	308

* Localities refer to Fig. 1.

[†] C, CIT; L, LDGO. Analytical procedures are described in refs 7 (CIT) and 15 (LDGO). Sr and Nd isotope analyses were performed in the same laboratory.[‡] $\epsilon_{\text{Nd}} = [({}^{143}\text{Nd}/{}^{144}\text{Nd})_{\text{sample}} / ({}^{143}\text{Nd}/{}^{144}\text{Nd})_{\text{bulk Earth}} - 1] \times 10^4$. For all analyses, ϵ_{Nd} of BCR-1 standard was taken to be -0.4 ; measured ${}^{143}\text{Nd}/{}^{144}\text{Nd}$ values were: CIT, 0.511826; LDGO, 0.512645. Direct comparison of samples: DSDP191, CIT, $+10.2$; LDGO, $+10.6$. For both CIT and LDGO, ${}^{87}\text{Sr}/{}^{86}\text{Sr}$ was normalized to ${}^{86}\text{Sr}/{}^{88}\text{Sr} = 0.1194$.

§ Quaternary lavas; A. Tsvetkov, personal communication.

said for the Samail ophiolite³⁰, but with much smaller ϵ_{Nd} variation, and for the Kanaga xenoliths and the older Attu volcanic rocks (Table 1).

Tectonic cross-sections

The brief (55 Myr)^{18,19} history of the Aleutian island arc records the juxtaposition of several geochemically distinct terranes: oceanic and back-arc lithosphere occurring either at the surface (Attu) or buried under younger arc magmas (Kanaga); continent-derived sediment, with ϵ_{Nd} values near zero⁷, retained within the arc crust or added to it by off-scraping or subcretion^{32,33}; and subduction-related magmas intruded along an arc axis which has migrated northward during the Tertiary (see Fig. 1). The proportion of these terranes varies along the arc: a western Aleutian cross-section (near Attu) will have a higher proportion of oceanic lithosphere than one through the central Aleutians (near Adak). Further east, along the Alaska Peninsula we expect additional terranes, including isotopically distinct older continental crust and exotic lithospheric fragments carried

from far to the south³⁴. Sediment draping the flanks of the arc contains a variable proportion of erupted and eroded arc material representing the exposed terranes.

Figure 3a is a tectonic cross-section of the central Aleutian arc¹, which applies more specifically to the Adak-Kanaga region. A second section (Fig. 3b), representing the Attu region (consistent with shallow, thrust-mechanism seismicity; Fig. 3c)^{35,36} looks quite different. Here, we postulate that structural thickening of the crust by nearly arc-parallel thrusting is necessary to elevate the back-arc oceanic crust now exposed at the surface. We find very little arc-related magmatism that could build the crust up from abyssal depth to sea level over the past 30 Myr.

Note, in Fig. 3a, that the oceanic or back-arc crustal section (and in particular the sediment associated with it) is completely recrystallized during arc-related deformation and heating. It has also been intruded by arc magmas (it was an arc magma that carried xenoliths of the section to the Earth's surface on Kanaga island). The arc magmas have variably contaminated the

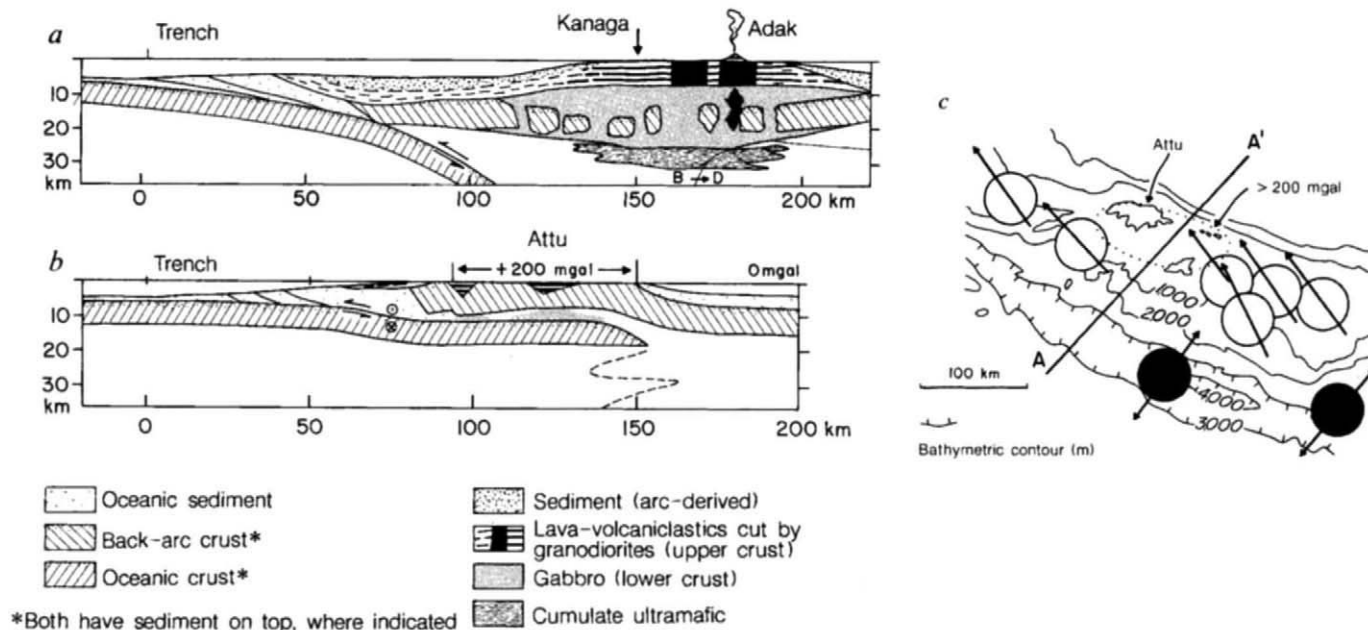


Fig. 3 *a, b*, Tectonic cross-sections of the central and western Aleutian arc (no vertical exaggeration). *a*, Section in the region of Adak and Kanaga (Fig. 1); near-surface tectonic features are after refs 44–46. Oceanic terranes are represented by symbols on the left, subduction-related terranes by those on the right. Geometry of lower-crustal terranes after ref. 1. Projected positions of Adak and Kanaga xenolith localities are shown, as is the present position of the brittle-ductile transition (B → D). Migration of the brittle-ductile transition away from the trench in the Tertiary is inferred from the deformation of mantle and lower crustal xenoliths from Kanaga island. *b*, Cross-section along A–A' in map *c*. Crustal imbrication is inferred from earthquake mechanisms, from the paucity of arc-related igneous rocks, and from the extensive sections of back-arc-related volcanic rocks exposed on the surface of the islands. *c*, Map view of Attu region, showing earthquake mechanisms^{35,36} and extent of +200 mgal free-air gravity anomaly³⁷.

xenoliths (and the deep crust of the arc), yet not to the extent of erasing their Nd isotope signature. From textural studies, it appears that some mineralogical vestiges of a pre-contamination condition exist^{26–29}.

How much crustal contamination of arc magmas by buried hydrothermally altered oceanic crust (including sediment) occurs? We may speculate that water makes melting relatively easy and that the buried oceanic crust should contribute disproportionately to the arc magma contamination. The constant ϵ_{Nd} (and $^{87}\text{Sr}/^{86}\text{Sr}$) of the Moffett cumulate-textured xenoliths (see Table 1) is important in this context, for with contamination, ϵ_{Nd} and $^{87}\text{Sr}/^{86}\text{Sr}$ should be dependent on fractionation stage (as they apparently are for the plutons). Despite our published opinion to the contrary^{4,14}, we suggest that the trapped oceanic crust may melt to yield the enigmatic, high- ϵ_{Nd} Mg-andesites, and may yield a high- ϵ_{Nd} component for Tertiary plutons.

In Fig. 3*b*, we correlate the shallow, thrust-mechanism earthquakes^{35,36} (Fig. 3*c*) with oblique intracrustal thrusting (parallel to the trench). We have indicated intracrustal thrusting at the interfaces between imbricated oceanic and back-arc crustal sections. These interfaces will act as strain guides, because of the presence of both water (hydrothermal alteration) and sediment. The interfaces are also the likely intrusion sites for the small amounts of arc-related intrusives. Shallow structural imbrication of oceanic and back-arc crust, (perhaps including oceanic plateaus) is consistent with a +200 mgal free-air gravity anomaly and the high gravity gradient in the region³⁷. Note that immediately west of Attu, the arc crust is very much thinner and deeper. In our interpretation, Attu represents a structural culmination of arc-parallel oblique thrusting.

Discussion

The identification of geochemically coherent terranes within the Aleutian arc lithosphere provides a rational and useful framework for consideration of the chemical controls on arc

tectonics ('chemotectonics'). The creation of mixed terranes by erosion and magmatic assimilation should also be well described within this framework, as should the comparison of the Aleutians and other arcs. The structural response of various levels in the arc lithosphere to the stress regimes within a convergent plate margin will be controlled by water, and to a lesser degree by rock type within the arc crust. We note specifically the potential role of trapped hydrothermally altered oceanic crust at mid-crustal levels and its potential role as a strain guide (see Fig. 3*b*). This makes plausible the coincidence of major geochemical and tectonic boundaries within the arc crust.

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- Kay, S. M. & Kay, R. W. *Geology* **13**, 461–464 (1985).
- Kay, S. M., Kay, R. W. & Citron, G. P. *J. geophys. Res.* **87**, 4051–4072 (1982).
- Kay, S. M. & Kay, R. W. *Contrib. Miner. Petrol.* **90**, 276–290 (1985).
- Kay, R. W. *J. Geol.* **88**, 497–522 (1980).
- Perfit, M. R. & Kay, R. W. *Geochim. cosmochim. Acta* **50**, 477–481 (1986).
- Morris, J. D. & Hart, S. R. *Geochim. cosmochim. Acta* **47**, 2015–2030 (1983).
- VonDrach, V., Marsh, B. D. & Wasserburg, G. J. *Contrib. Miner. Petrol.* **92**, 13–34 (1986).
- White, W. H. & Patchett, J. *Earth planet. Sci. Lett.* **67**, 167–185 (1984).
- Conrad, W. K. & Kay, R. W. *J. Petrol.* **25**, 88–125 (1984).
- Tera, F. *et al.* *Geochim. cosmochim. Acta* (in the press).
- DeBari, S., Kay, S. M. & Kay, R. W. *J. Geol.* (in the press).
- Swanson, S., Kay, S. M., Brearley, M. & Scarfe, C. in *Manila Xenoliths* (ed. Nixon, P. H.) (Wiley, New York, in the press).
- Kay, R. W., Sun, S.-S. & Lee-Hu, C.-N. *Geochim. cosmochim. Acta* **42**, 263–273 (1978).
- Kay, R. W. *J. Volcanol. geotherm. Res.* **4**, 117–132 (1978).
- Goldstein, S. thesis, Columbia Univ. (1985).
- Borsuk, A. M. & Tsvetkov, A. A. *Int. Geol. Rev.* **24**, 317–327 (1982).
- Kay, R. W., Rubenstone, J. L. & Kay, S. M. *Abstr. 27th int. geol. Congr.* Vol. 3, 252 (Izdatsel'stvo Nauka, Moscow, 1984).
- Rubenstone, J. L. thesis, Cornell Univ. (1984).
- Scholl, D. W., Vallier, T. L. & Stevenson, A. J. *Geology* **14**, 43–47 (1986).
- Cox, A. & Engebreton, D. *Nature* **313**, 472–474 (1985).
- Gates, O., Powers, H. A. & Wilcox, R. F. *Bull. U.S. geol. Surv.* **1028U**, 709–822 (1971).

22. Engebretson, D., Cox, A. & Gordon, R. *J. geophys. Res.* **89**, 10291-10310 (1984).
23. Rea, D. & Duncan, R. A. *Geology* **14**, 373-376 (1986).
24. McCulloch, M. T. & Perfit, M. R. *Earth planet. Sci. Lett.* **56**, 167-179 (1981).
25. DeLong, S. E., Hodges, F. N. & Arculus, R. J. *J. Geol.* **83**, 721-736 (1975).
26. Johnston, L. thesis, Cornell Univ. (1983).
27. Pope, R. thesis, Cornell Univ. (1983).
28. Kay, S. M., Johnston, L. & Kay, R. W. *Geol. Soc. Am. Abstr. Progm.* **15**, 603 (1983).
29. Pope, R. S., Kay, S. M. & Kay, R. W. *Eos* **62**, 1092 (1981).
30. McCulloch, M. T., Gregory, R. T., Wasserburg, G. J. & Taylor, H. P. *Earth planet. Sci. Lett.* **46**, 201-211 (1980).
31. McCulloch, M. T. & Cameron, W. E. *Geology* **11**, 727-731 (1983).
32. Karig, D. L. & Kay, R. W. *Phil. Trans. R. Soc. A* **301**, 233-251 (1981).
33. Kay, R. W. *Tectonophysics* **112**, 1-15 (1985).
34. Stone, D. B. & Packer, D. R. *Tectonophysics* **37**, 183-201 (1977).
35. Cormier, V. *Geol. Soc. Am. Bull.* **86**, 443-453 (1975).
36. Newberry, J., LaClair, D. L. & Fujita, K. *J. Geodyn.* (in the press).
37. Bowin, C., Warsi, W. & Milligan, J. *Geol. Soc. Am. Map Ser.* MC-45 (1981).
38. Kay, R. W. & Hubbard, N. J. *Earth planet. Sci. Lett.* **38**, 95-116 (1978).
39. Kay, S. M., Kay, R. W., Bruecker, H. K. & Rubenstone, M. L. *Contr. Miner. Petrol.* **82**, 99-116 (1983).
40. Fraser, G. D. & Snyder, G. L. *Bull. U.S. geol. Surv.* **1028-M**, 371-408 (1959).
41. Citron, G. thesis, Cornell Univ. (1980).
42. DeLong, S. E., Perfit, M. R., McCulloch, M. T. & Ach, J. *J. Geol.* **93**, 609-618 (1985).
43. Nye, C. & Reid, M. *J. geophys. Res.* (in the press).
44. Scholl, D. W., Vallier, T. L. & Stevenson, A. J. *Am. Mem. Am. Ass. Petrol. Geol.* **34**, 413-439 (1983).
45. Scholl, D. W., Vallier, T. L. & Stevenson, A. J. *Geology* **14**, 43-47 (1986).
46. Engdahl, E. R. & Billington, S. *Bull. seism. Soc. Am.* **76**, 77-93 (1986).

Bovine papillomavirus genome elicits skin tumours in transgenic mice

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Transmission of the bovine papillomavirus-1 (BPV-1) genome through the mouse germ line results in the heritable formation of fibropapillomas of the skin, a tissue-specific phenotype analogous to that observed in natural BPV-1 infection of cattle. Oncogenesis is slow, with tumours first arising at 8-9 months of age, usually in areas prone to wounding. Extrachromosomal BPV-1 DNA is detected in all tumours, whereas normal tissues show only integrated DNA.

BOVINE papillomavirus 1 (BPV-1) is an icosahedral virus which infects cutaneous tissues of cattle and induces benign fibropapillomas with proliferative squamous epithelial and dermal fibroblast components^{1,2}. The BPV-1 genome is circular double-stranded DNA of ~8 kilobases (kb). The virus infects both dermal fibroblasts and epidermal epithelial cells, where the BPV-1 genome replicates as an autonomous plasmid, inducing proliferation of both cell types. Virus particles are produced only in terminally differentiated epidermal keratinocytes, where the BPV-1 DNA replicates to a very high copy number (about 10,000 copies per cell). In contrast, the copy numbers range from 50 to 500 per cell in the proliferating dermal fibroblasts and in the squamous epithelial cells of the lower epidermis. BPV-1 shows a broad experimental host range; it has been shown to infect horses, hamsters and one inbred strain of mouse (C3H₁₀₁B). Infection of these heterologous species results in the development of fibroblastic tumours, generally at the site of infection/inoculation, but without the subsequent production of infectious virus particles.

The genetics of BPV-1 replication and transformation have been studied principally in cultured murine cells^{1,2}. BPV-1 replicates as a stable extrachromosomal element in a variety of cultured mouse fibroblasts, and is able to morphologically transform a cell line (C127) derived from a murine mammary tumour, as well as NIH 3T3 cells. The necessary *cis*- and *trans*-acting elements for both transformation and replication have been localized to a fragment which comprises 69% of the viral genome, given that the genetic assay is restricted to a few established cell lines.

In the present study we have sought to address the properties of the BPV-1 genome when transmitted through the mouse germ line, in order to assess the ability of BPV to replicate in and transform murine cells in the context of its stable presence in every cell of the animal.

Generation of transgenic mice

The bovine papillomavirus genome was injected into fertilized mouse embryos as a *SalI*-linearized plasmid clone, which is shown in Fig. 1. The plasmid (called pBPV1.69) carries a partial tandem duplication of the complete BPV-1 genome. This configuration was used for two reasons: first, to provide an

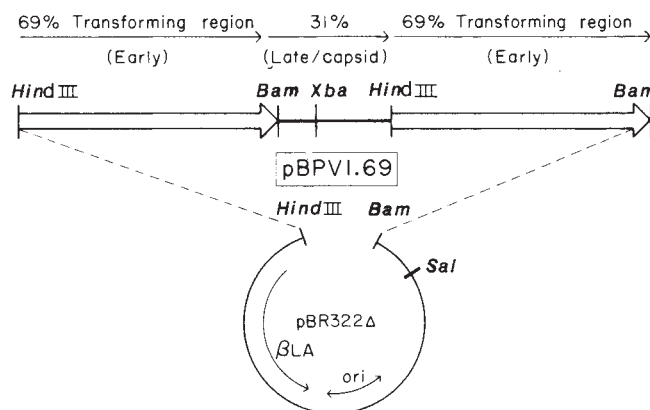


Fig. 1 Structure of the plasmid pBPV1.69. Two copies of the 69% transforming region (*HindIII*-*Bam*) of BPV-1 are separated by the 31% 'late' region, thereby generating a reiterated copy of the intact BPV-1 genome—a 100% fragment plus an additional copy of the 69% transforming region.

Methods. The plasmid pBPV1.69 was linearized by digestion with *SalI*, purified by phenol and chloroform/isoamyl alcohol extraction, and ethanol precipitation. The concentration was adjusted to ~1,000 ng ml⁻¹ before injection into fertilized one-cell embryos, which were implanted into pseudopregnant female mice and allowed to develop. The embryos were derived from intercrosses of (C57BL/6J × DBA/2J)F₁ hybrid mice, and thus were F₂ hybrids. The one transgenic mouse (M120) which survived to sexual maturity was backcrossed to C57BL/6J females, and subsequent generations were either intercrossed to derive homozygotes, or backcrossed to C57BL/6J mice. The microinjections, embryo transfers and DNA analyses were performed essentially as described elsewhere¹⁹.

uninterrupted colinear copy of the genome, as is encountered in its normal circular configuration; second, to examine the possibility that BPV-1 could excise and replicate as an extrachromosomal element. Experiments with integrated simian virus 40 (SV40) genomes have demonstrated that partial tandem duplications facilitate precise excision by homologous recombination in conditions permissive for SV40 replication³⁻⁵. A pre-

vious study showed that when circular BPV-1 DNA was injected into mouse embryos, the transgenic mice which arose carried only integrated forms (F. Costantini, personal communication), suggesting that early mouse embryos are nonpermissive for BPV-1 plasmid replication. Thus in the present study we intended to create transgenic mice carrying integrated BPV-1 DNA in the germ line, but with a topology that might allow excision and extrachromosomal replication in somatic tissues.

Two transgenic mice arose from embryos injected with the linearized pBPV1.69 plasmid. One mouse (116) was born with limb deformities and died ~36 h after birth, and will not be discussed further. The second mouse (120) carried about five copies of the BPV1.69 plasmid, integrated in a head-to-tail tandem array. This mouse developed normally, was fertile, and transmitted the acquired DNA as a single insertion in a mendelian fashion. Homozygotes were derived from intercross mating of siblings. No abnormalities were observed in either heterozygotes or homozygotes during the early breeding analysis.

Development of cutaneous tumours

Transgenic mice harbouring the pBPV1.69 insertion are phenotypically normal during development and early adult life. However, skin tumours begin to appear as the mice grow older. Tumours are first apparent at about 8 months of age, arise in multiple locations on the body, and eventually develop in almost every transgenic mouse in this lineage. In addition to obvious protuberant growths, large areas of the skin become abnormal, with thickening, hardening, and marked hair loss. Small wart-like protrusions are often scattered throughout areas of the abnormal skin. Tumours are most frequent in the face and head area, and on the ends of the tails of heterozygous mice, which were clipped at 3 weeks of age for DNA analysis. The tumours are initially benign, but can become malignant and locally invasive, although they have not been found to metastasize to other tissues. No examples of primary tumours arising in internal organs have been observed. Occasionally, mice become severely affected, with most of the skin becoming grossly abnormal, and multiple protuberant tumours developing on the face, neck, mouth, body and tail. There have been no examples of non-transgenic mice developing skin tumours, even following extended cohabitation with affected transgenic mice, which suggests that infectious BPV-1 virus is not being produced. This conclusion is supported by immunohistochemical analyses of affected tissues using antisera that recognize the BPV-1 capsid proteins, which have failed to detect these virion components (P. Howley, personal communication). Representative examples of the skin tumours which arise in these transgenic mice are shown in ref. 20.

Tumour pathology

Various protuberant tumours and abnormal skin were analysed after formalin fixation, standard tissue sectioning, and histochemical staining with haematoxylin and eosin: four examples are shown in Fig. 2, together with normal skin. In all cases, the affected tissues contained proliferating dermal fibroblasts, in rather disorganized swarms of densely packed cells. The obvious proliferation and disorganization of the dermal layer is characteristic of all tumours examined, and is almost always the major component of affected tissues. This characteristic is similar to that observed in dermatofibromas and fibromatoses which arise in humans⁶.

There are two different histopathologies observed in the epidermis of the affected tissues. Abnormal skin shows a grossly thickened dermal layer and an atrophic epidermis, with significant loss and disorganization of hair follicles and glands. The epidermis is only a few cells thick. One can speculate that the thickened and disorganized dermis (or the transformed state of the dermal fibroblasts) may somehow be impaired in its ability to provide the proper stratum or the required nutrients and growth factors for normal epidermal cell growth. Abnormal skin

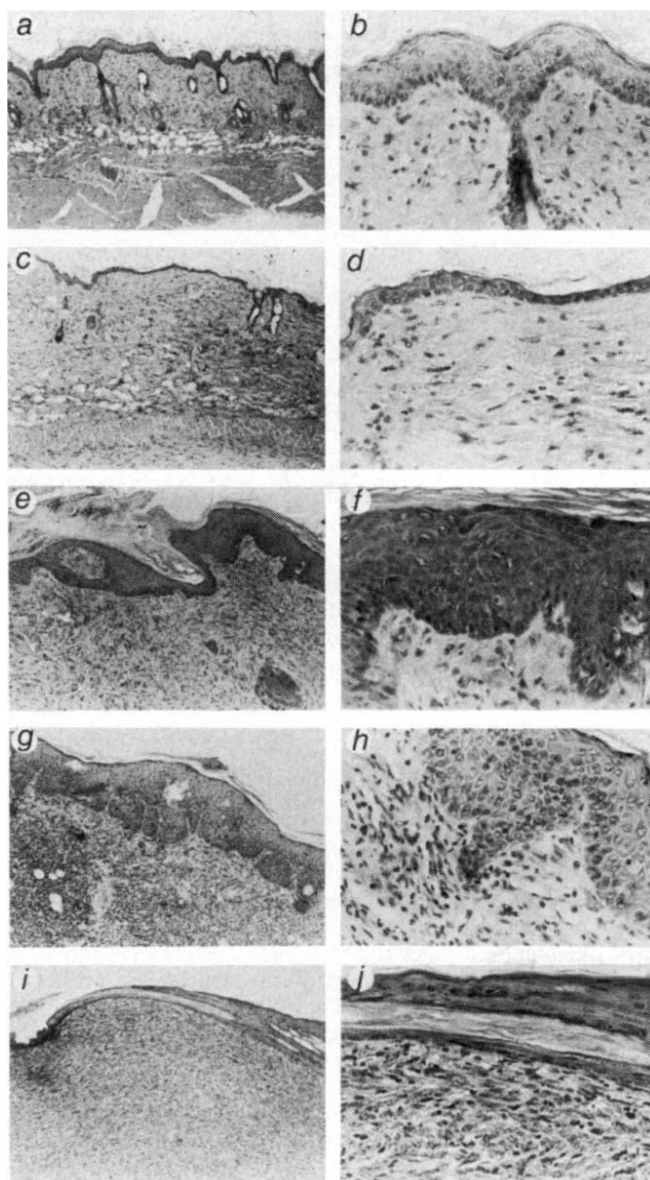


Fig. 2 Histological analysis of affected cutaneous tissue. In each case the tissue is shown at two magnifications, $\times 30$ (left) and $\times 120$ (right). *a, b*, Normal skin, with hair follicles and glands embedded in the dermis, and an epidermal layer of average size for body skin. *c, d*, Abnormal skin, showing hyperplasia of the dermal fibroblasts and thinning of the overlying epidermis, with loss of hair follicles and glands. *e, f*, Tumour at the tip of a tail, showing a disorganized and enlarged mass of dermal fibroblasts, and a hyperplastic epidermal layer. *g, h*, Facial tumour, with dermal fibroblast proliferation and thickening of the overlying epidermis. *i, j*, An anaplastic tumour, which arose on a hingleg, with multi-nucleated cells that are not readily identifiable as either dermal fibroblasts or epidermal epithelial cells. Tissues were excised and fixed in 10% formalin, after which they were embedded in paraffin, and thin sections taken. The sections were stained with haematoxylin and eosin in a standard histochemical process.

also shows scattered small wart-like protrusions, which appear to be focal proliferations of melanocytes characteristic of blue nevus⁶.

The second class of abnormality seen in the epidermis is associated with protuberant tumours, where the epidermis shows considerable hyperplasia. The layer of keratinocytes appears unusually thick, and the cell types may be disorganized as well. Although the extent of epidermal hyperplasia is significant, it is less than the degree of dermal fibroblast proliferation. This

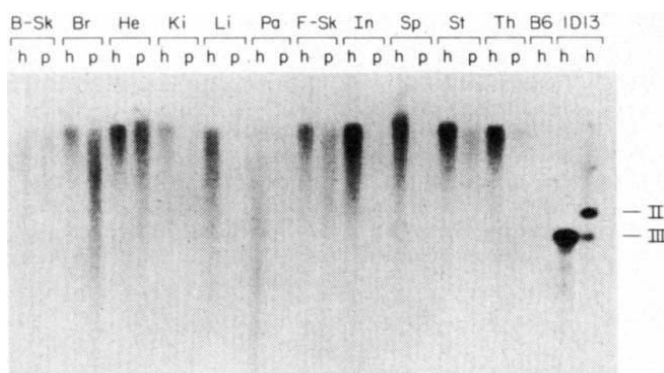


Fig. 3 BPV DNA in the tissues of a young mouse. Total DNA was isolated from a series of tissues, and fractionated into a high- M_r spooled portion (h) and a pellet fraction (p) composed of the low- M_r DNA which did not spool. The DNAs were digested with *SacI*, which does not cut the pBPV1.69 plasmid. The DNAs were electrophoresed through a 0.5% agarose gel, transferred to nitrocellulose, and hybridized to a radiolabelled BPV plasmid. The tissues analysed are body skin (B-Sk), brain (Br), heart (He), kidney (Ki), liver (Li), pancreas (Pa), facial skin (F-Sk), small intestine (In), spleen (Sp), stomach (St) and thymus (Th). Additional tissues examined but not shown include testes, tail, body wall, lung and skeletal muscle. ID13 is a BPV-transformed C127 cell line containing ~400 copies of episomal BPV DNA. B6 is C57BL/6J DNA (from a normal mouse, which lacks BPV sequences). The positions of the relaxed circular and linear forms of extrachromosomal BPV are indicated (forms II and III, respectively). High- M_r ID13 DNA (h) was digested with *Xba* (left- or *Sac* (right), to either linearize the resident BPV genome or leave it untouched.

is in contrast to naturally occurring fibropapillomas in cattle, where the dermal fibroblast and epidermal components are approximately equally represented. There are several possible explanations for this difference. One is that the penetrance of the BPV-1 oncogenes is different in two cell types, being more effective in the dermal fibroblasts than in the squamous epithelial cells; this assumes that the epithelial proliferation is a direct result of BPV-1 gene expression. A second possibility, however, is that the epidermal response is reactive to the dermal proliferation, and is not induced directly by a BPV-1-encoded function. Conversely, a third possibility is that BPV-1 gene expression in the epidermis is directing the fibroblast proliferation. The examination of these possibilities will require separate analysis of the two tissue layers.

BPV-1 genome in normal and tumour tissues

The structure and form of the BPV-1 genome has been examined in a variety of tissues in both young normal mice and in older tumour-bearing mice. In view of the possibility that BPV excises and replicates as an episome, the analysis was designed to detect free copies of the BPV genome. DNA from each tissue was separated into low- and high-relative molecular mass (M_r) fractions, which were then digested with restriction enzymes that cleave the BPV-1 genome either once (*XbaI*) or not at all (*SacI*). The *SacI* digestion should leave the pBPV1.69 plasmid in a fragment of very high M_r (>85 kb), composed of the head-to-tail tandem array (of about five copies) that was produced during integration after microinjection of the original embryo. In contrast, free copies of BPV-1 DNA should migrate on gels in the 8-kb range.

Figure 3 shows an example of a DNA blotting analysis of tissues in a young mouse, ~6 weeks old. No hybridizing bands were observed in the *SacI* digests of either high- or low- M_r fractions of any tissue. In comparison, the ID13 cell line, which is transformed by BPV DNA, shows 8-kb bands, demonstrating the presence of extrachromosomal DNA. This and similar analy-

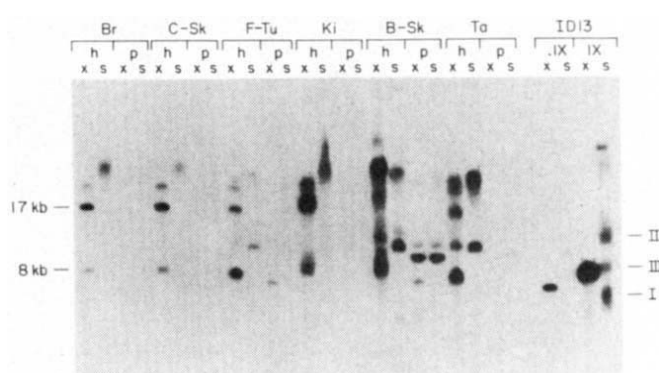


Fig. 4 BPV DNA in a tumour-bearing mouse. Mouse 907 carried a facial tumour, a tail tumour growth and an area of affected skin, with substantial hair loss and dermal thickening. Tissues were collected, and DNAs extracted and analysed as described in Fig. 3 legend. The tissues analysed are brain (Br), facial tumour (F-Tu), cheek skin near the facial tumour (C-Sk), kidney (Ki), abnormal body skin (B-Sk), and tail with tumour (Ta). Both spooled (h) and pellet (p) fractions were digested with either *XbaI* (X) or *SacI* (S). Kidney DNA was applied to this analysis in a fourfold greater amount than the other tissues examined, to verify the lack of appreciable rearranged or extrachromosomal DNA in normal tissue. The positions of the three forms of extrachromosomal BPV-1 in ID13 cells are indicated by I (supercoiled), II (relaxed circular) and III (linear). Digestion with *XbaI*, which cuts once within the 31% fragment of BPV, should release the full-length 17-kb pBPV1.69 plasmid from the integrated concatenate, as well as linearize the 100% BPV circular form (7.9 kb) that would be released by homologous recombination across the reiteration in the plasmid. The 8-Kb band in the *Xba* digests of brain and cheek skin represents a rearranged copy of the input 17-kb BPV 1.69 plasmid that releases this fragment (the 100% BPV genome) from the integrated concatenate (data not shown), which was apparently produced by recombination between injected molecules during integration.

ses have shown that the BPV genome does not undergo detectable excision events in the tissues of young transgenic mice, including the skin, where tumours will arise in later life. When these same DNAs were digested with restriction enzymes that cleave the pBPV1.69 plasmid, an identical pattern was observed in every tissue; this pattern is consistent with the plasmid being present as a stable head-to-tail concatenate in high- M_r DNA (not shown).

In contrast, the analyses of mice bearing tumours revealed rearrangements in the BPV genome in all affected tissues. Figure 4 presents the analysis of one such mouse. The low M_r DNA fractions in a facial tumour, a tail tumour and abnormal skin all show free copies of BPV-1. Comparison with the unit-length BPV-1 genome maintained in cultured mouse C127 cells indicated that the episomes are predominantly full-length BPV, presumably arising from precise excision from the integrated DNA. Most of the extrachromosomal copies migrated as relaxed circular or linear DNAs (forms II and III, respectively). It is not clear whether this represents the authentic condition in the tumour cells, or rather results from the DNA isolation and *SacI* digestion, which was performed to facilitate migration of high- M_r DNA in the agarose gel. Some DNAs showed additional bands which may represent the excision of the BPV/plasmid recombinant, which is also duplicated as a consequence of the head-to-tail integration observed in early embryos. The spooled high- M_r fractions of the affected tissues also showed amplified BPV DNA. In skin, it seems that both free copies and *in situ* integrated copies contribute to the amplification. Again, normal tissues, including facial skin, show no amplified, rearranged or

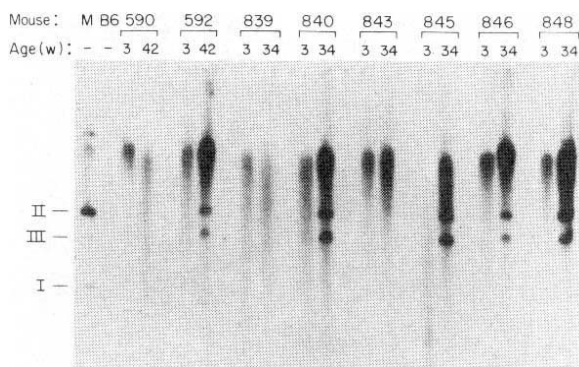


Fig. 5 Correlation between tumours and episomes. Tail DNAs were isolated from eight mice at both 3 weeks and 9–10 months old. Five of these mice (592, 840, 845, 846 and 848) had a tumour on the tip of the tail, while the other three appeared normal. The DNAs were digested with *Sac*I and analysed as described for Figs 3, 4. The marker lane (M) contains DNA from the ID13 cell line, which contains episomal BPV: the supercoiled, relaxed circular and linear forms are indicated. B6 is C57BL/6J DNA, which does not contain BPV sequences.

free DNA. This was particularly apparent in the analysis of the kidney DNA, which was used at about a fourfold higher DNA concentration. Examination of the *Sac*I digest revealed no extrachromosomal copies of the BPV-1 genome, and the *Xba*I digest was comparable to (although more intense than) brain or normal skin.

Analysis of a number of affected mice bearing protuberant tumours and abnormal skin has revealed a correspondence between the tumours and the presence of free copies of the BPV-1 genome in the DNAs of those tumours; a pertinent example is shown in Fig. 5, in which the tail DNAs of several mice are analysed. The tails were originally clipped at 3 weeks old, to assess transmission of the transgene. At approximately 10 months of age, an additional portion of the tail from each mouse was removed, and the DNAs were subjected to the blotting analysis described above. Five mice were seen to have free copies of the BPV-1 genome in their tail tissue at 10 months of age, while none had any detectable levels at 3 weeks. These five mice had tumour growth on the tips of their tails, while their siblings did not. As in the results shown in Fig. 4, again there was a correspondence between free copies of the BPV-1 genome and the presence of abnormal tissue.

Genetic aspects of tumour development

The characteristics of tumour formation suggest that these transgenic mice will be useful in the study of secondary events which participate in oncogenesis. The presence of the BPV oncogenes is apparently insufficient, in that the tumours arise after a long latency. Furthermore, tumours occur most frequently in areas prone to wounding. There is precedent for irritation and wounding as cofactors in a variety of cancers, including BPV-induced fibropapillomas in cows^{1,2}, crown gall tumours in plants⁷, and Rous sarcoma virus-induced sarcomas in chickens⁸, as well as in experimental animal models of tumour progression^{9–11}.

Thus, the BPV-1 genome can be considered to be a genetic predisposition to the induction of fibropapillomas of the skin, one which requires additional events before oncogenesis occurs. One prospective event might be the transcriptional activation of a dormant BPV genome, as this has been observed both in studies of BPV-1 transformation of cultured cells¹², and in

oncogenesis induced through persistent papillomavirus infection of the Brazilian rat¹³. In addition, there is a high incidence of BPV-1-induced tumours in cows grazing on bracken fern, which is tumorigenic in laboratory animals, and which may be acting as a cofactor in this natural oncogenesis¹⁴. The ability of carcinogens¹⁵ or prospective cellular (growth) factors¹⁶ to complement the BPV oncogenes in tumour development can be assayed directly using these transgenic mice.

In a previous study (R. Palmiter and R. Brinster, personal communication), 15 lines of transgenic mice harbouring the BPV 69% transforming region were generated and maintained for 10 months. No abnormalities were observed in any of these lineages, which suggests that either the topology of the BPV1.69 plasmid or the presence of the 31% portion of the BPV genome is important for the phenotype described here. Consistent with this possibility is the observation of Sarver *et al.*¹⁷ that the complete genome is more efficient at transforming C127 cells than is the 69% fragment, as well as the recent studies of Lusky and Botchan¹⁸, which demonstrated that the 31% region contains a *cis*-acting element that stimulates efficient BPV plasmid replication in cultured cells.

Thus, transgenic mice provide a new model in which to study the tissue specificity of BPV gene expression, the activities of the BPV oncogenes, and the genetic requirements of the BPV-1 genome in eliciting neoplasia—in particular, the implications that either the 31% region, extrachromosomal replication, or gene amplification could be important for oncogenesis. The present results also presage similar application of this approach to human papillomavirus genomes.

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1. Lancaster, W. D. & Olson, C. *Microbiol. Rev.* **46**, 191–207 (1982).
2. Pfister, H. *Rev. Physiol. Biochem. Pharmac.* **99**, 111–181 (1984).
3. Botchan, M., Topp, W. & Sambrook, J. *Cold Spring Harb. Symp. quant. Biol.* **43**, 709–719 (1979).
4. Schaffner, W., Topp, W. & Botchan, M. *Alfred Benzon Symp.* **13**, 191–203 (1979).
5. Hanahan, D., Lane, D., Lipsich, L., Wigler, M. & Botchan, M. *Cell* **21**, 127–139 (1980).
6. Lever, W. F. & Schaumburg-Lever, G. *Histopathology of the Skin* (Lippincott, London, 1983).
7. Stachel, S. E., Messens, E., Van Montagu, M. & Zambryski, P. *Nature* **318**, 624–629 (1985).
8. Dolberg, D. S., Hollingsworth, R., Hertle, M. & Bissell, M. J. *Science* **230**, 676–678 (1985).
9. Pozharisski, K. M. *Cancer Res.* **38**, 3824–3830 (1975).
10. Marks, F., Bertsch, S., Grimm, W. & Schweizer, J. *Carcinogenesis* Vol. 2 (eds Slaga, T. J., Sivak, A. & Boutwell, R. K.) 97–116 (Raven, New York, 1978).

11. Konstantinidis, O. A., Smulow, J. B. & Sonnenschein, C. *Science* **216**, 1235–1237 (1982).
12. Amtmann, E. & Sauer, G. *Nature* **296**, 675–677 (1982).
13. Amtmann, E., Volm, M. & Wayss, K. *Nature* **308**, 291–292 (1984).
14. Jarrett, W. F. H., McNeil, P. E., Grimshaw, W. T. R., Selman, I. E. & McIntyre, W. I. M. *Nature* **274**, 215–217 (1978).
15. Hecker, E., Fusenig, N. E., Kunz, W., Marks, F. & Theilmann, H. W. (eds) *Co-Carcinogenesis and Biological Effects of Tumor Promoters* (Raven, New York, 1982).
16. Sporn, M. B. & Roberts, A. *Nature* **313**, 745–747 (1985).
17. Sarver, N., Bryne, J.-C. & Howley, P. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7147–7151 (1982).
18. Lusky, M. & Botchan, M. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3609–3613 (1986).
19. Hogan, B., Costantini, F. & Lacy, L. *Manipulating the Mouse Embryo—A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1986).
20. Hanahan, D., Alpert, S. & Lacey, M. *Alfred Benzon Symp.* **24** (in the press).

Looking for cosmic strings

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Cosmic strings predicted in some grand unified theories can produce double images of distant galaxies and quasars¹⁻³, and it has been argued that some of the known double quasars may be due to strings^{2,4}. To test this hypothesis, one can look for other characteristic effects of strings in the vicinity of double quasars, such as a discontinuous change of the microwave background temperature across the string^{3,5}, lines of double galaxies², and images of galaxies with a sharp edge⁴. In most of the previous work on the gravitational effects of strings it was assumed either that the string is static or that both the string and its velocity are perpendicular to the line of sight. Here I show that in more general configurations the expressions for the angular separation of double images and for the temperature discontinuity $\delta T/T$ across the string are modified by trigonometric and relativistic factors. More importantly, the line connecting the double images is not, in general, perpendicular to the apparent direction of the string (contrary to what one would naively expect). Although the analysis presented here is rather elementary, the results may nevertheless be useful for interpreting the observations.

We first consider the situation in the rest frame of the string. The metric of a straight static string is a conical space^{1,3}. It is locally flat, but has an angle deficit

$$\delta = 8\pi G\mu \quad (1)$$

where G is the gravitational constant and μ is the mass per unit length of string. This space can be obtained from a euclidean space by cutting out a wedge of angular width δ and identifying the exposed surfaces. The existence of strings can be consistent with the isotropy of the cosmic microwave background only if⁵ $G\mu < 10^{-5}$, and we shall assume this to be the case. The angular separation of double images due to a string is given by^{2,3}

$$\delta\varphi_0 = l(d+l)^{-1}\delta \sin \theta \quad (2)$$

where d and l are the normal distances from the observer and from the quasar to the string and θ is the angle between the string and the line of sight.

Strings are expected to move at relativistic speeds, the mean square velocity (in units of c , the speed of light) being $\langle v^2 \rangle \approx 0.5$ (with respect to local co-moving frames). Because only transverse motion of strings is observable, we shall assume that $\mathbf{v}$ is perpendicular to the direction of the string. To find the angular separation between the images in the observer's frame, let $\mathbf{k}$ and $\mathbf{k}'$ be the four-dimensional wave vectors of the light waves (with frequencies ω and ω') corresponding to the two images, and consider the invariant $\mathbf{k} \cdot \mathbf{k}' = \omega\omega'[1 - \cos(\delta\varphi)] \approx 0.5 \omega\omega'(\delta\varphi)^2$.

In the rest frame of the string, S_0 , the two frequencies are equal: $\omega = \omega' = \omega_0$. In the observer's frame, S , ω and ω' are slightly different because of the difference in the directions of $\mathbf{k}$ and $\mathbf{k}'$. However, the difference is of the order of $\delta\varphi$ and can be neglected to the lowest order in $G\mu$; thus, $\omega\delta\varphi = \omega_0\delta\varphi_0$. The frequencies ω and ω_0 are related by $\omega = \gamma(1 - \mathbf{n} \cdot \mathbf{v})\omega_0$, where $\mathbf{n}$ is the direction from the observer to the quasar, $\mathbf{v}$ is the velocity of the string (that is, the velocity of S_0 relative to S) and $\gamma = (1 - v^2)^{-1/2}$. Hence,

$$\delta\varphi = \gamma^{-1}(1 - \mathbf{n} \cdot \mathbf{v})^{-1}\delta\varphi_0 \quad (3)$$

Depending on the relative directions of $\mathbf{v}$ and $\mathbf{n}$, the angular separation has a value in the range $\kappa^{1/2}\delta\varphi_0 < \delta\varphi < \kappa^{-1/2}\delta\varphi_0$, where $\kappa = (1 - v)/(1 + v)$. Note that long strings are expected

to be at large redshifts, and hence to have a tendency to move away from us. (Here and below, I ignore the complications introduced by space-time curvature.)

The temperature discontinuity across the string arises because the relative velocity of two objects in a conical space is double-valued⁵: the answer depends on which way one parallel-transports the velocities around the string. In the rest frame of the string, S_0 , the observer moves past the string with velocity $-\mathbf{v}$. If the observer is at rest with respect to the sources of radiation on one side of the string, then the velocity of the sources on the other side of the string is $-\mathbf{v} + \mathbf{u}_0$, where $\mathbf{u}_0 = 8\pi G\mu \mathbf{v} \times \mathbf{s}$ and $\mathbf{s}$ is a unit vector along the string. The corresponding velocity difference in the observer's frame S is $\mathbf{u} = \gamma\mathbf{u}_0$. (Here I have expanded $\mathbf{u}$ to the lowest order in $G\mu$ and used the fact that $\mathbf{u}_0 \cdot \mathbf{v} = 0$.) The temperature discontinuity can be found from the Doppler formula, $\delta T/T = \mathbf{u} \cdot \mathbf{n}$, where $\mathbf{n}$ is a unit vector along the line of sight; thus,

$$\delta T/T = 8\pi G\mu \gamma \mathbf{n} \cdot (\mathbf{v} \times \mathbf{s}) \quad (4)$$

This expression has been derived in a different way by Vachaspati⁶.

If a segment of rapidly moving string is not perpendicular to the line of sight, then the observer sees more distant parts of the string with a retardation, and the apparent direction of the string differs from its actual direction. For simplicity, I shall consider only the case when the velocity of the string is perpendicular to the line of sight. Consider a coordinate system with y -axis along the line of sight and z -axis along the velocity of the string, so that the orientation of the string is parallel to the x - y plane. The observer sees a projection of the string on the x - z plane. Two points of the string with x -coordinate difference Δx are seen with a relative time delay $\Delta t = \Delta x \cot \theta$; hence, the observed difference in z -coordinates is $\Delta z = v\Delta t = (v \cot \theta)\Delta x$. The angle χ between the apparent and the actual direction of the string can be found from

$$\tan \chi = v \cot \theta \quad (5)$$

If there is a double quasar due to this string, then the line connecting the two images is along the z -axis and is not, in general, perpendicular to the apparent direction of the string (which can be observed, for example, by detecting the microwave temperature discontinuity).

Equation (5) applies to a relatively short segment of string subtending a small angle from the position of the observer. If an infinite straight string moves past the observer, he sees its distant parts with a retardation, so that the string appears to him to be curved. In the rest frame of the string, S_0 , light rays received at the origin at time $t_0 = 0$ were emitted from the string at space-time points

$$x_0^\mu = \{-(a^2 + \sigma^2)^{1/2}, \mathbf{a} + \sigma\mathbf{s}\} \quad (6)$$

Here, $\mathbf{a}$ is the normal vector from the origin to the string, $\mathbf{s} \perp \mathbf{a}$ is the unit vector along the string and σ is a parameter on the string (equal to the length along the string measured from the end of vector $\mathbf{a}$). We introduce a coordinate system with x -axis parallel to the string and z -axis along the velocity of the string in the observer's frame, S , and assume that the origins of S_0 and S momentarily coincide at $t = t_0 = 0$. The coordinates of the emission events in frame S are found from equation (6) by a Lorentz transformation; the spatial components are given by $x = \sigma$, $y = a_y$, $z = \gamma[a_z - v(a^2 + \sigma^2)^{1/2}]$, where $a^2 = a_x^2 + a_z^2$. To represent the apparent shape of the string, this three-dimensional curve must be projected onto a plane. A central projection from the origin to the plane $y = a_y$ gives

$$z = \gamma v[a - (a^2 + x^2)^{1/2}] \quad (7)$$

where I have set $\alpha_z = va$, so that $z(x=0) = 0$. Hence, a rapidly

moving infinite straight string appears to have the shape of a hyperbola.

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1. Vilenkin, A. *Phys. Rev. D* **23**, 852-857 (1981).
2. Vilenkin, A. *Astrophys. J.* **282**, L51-L53 (1984).
3. Gott, J. R. *Astrophys. J.* **288**, 422-427 (1985).
4. Paczynski, B. *Nature* **319**, 567-568 (1986).
5. Kaiser, N. & Stebbins, A. *Nature* **310**, 391-393 (1984).
6. Vachaspati, T. *Nucl. Phys. B* (in the press).

Cooling flows in ellipticals and the nature of radio galaxies

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One of the great puzzles in the study of active galaxies is the relationship between the properties of the nucleus and the large-scale morphology of the host galaxy. Galaxies which produce extended radio sources but little optical, ultraviolet or X-ray emission are invariably giant ellipticals or cD galaxies, whereas spirals tend to host 'optically active' nuclei, such as Seyferts¹ and at least some quasars^{2,3}. Rees and co-workers⁴⁻⁶ have interpreted this difference in radiative efficiency as a manifestation of the accretion flow close to the central black hole. But why should the mode of accretion at several Schwarzschild radii depend on the large-scale morphology of the host galaxy? I argue here that the connection arises from the manner in which interstellar gas is fed into the nucleus.

According to the scheme proposed by Rees *et al.*⁴, radio galaxies possess tenuous 'ion tori' which produce little radiation at optical, ultraviolet and X-ray wavelengths, whereas quasars and Seyfert galaxies possess optically thick accretion flows which radiate copiously. The mode of accretion depends on the ratio $\dot{m}$ of the accretion rate to the 'critical' accretion rate L_E/c^2 , where L_E is the Eddington limit and c is the speed of light. Given a viscosity parameter α (ref. 7), the flow is always a gas-pressure-supported 'ion torus' for $\dot{m} < 10^{-7} \alpha^2$ and is either a standard thin accretion disk ($\dot{m} < 10$) or a 'radiation torus' ($\dot{m} > 10$) for $\dot{m} > 50 \alpha^2$. For intermediate values of $\dot{m}$, however, the flow may assume one of two possible states: a thin disk or an ion torus. The flow pattern adopted probably depends on the manner in which gas is fed into the nucleus. In a spiral galaxy, the bulk of the interstellar medium resides in a disk, and it is plausible that a radiatively efficient flow pattern persists all the way to the black hole. Thus it seems natural that spiral galaxies should produce optically active galactic nuclei.

The situation is quite different for elliptical galaxies, which are permeated by a hot interstellar medium at a temperature close to the virial temperature of the galaxy⁸⁻¹⁰. X-ray measurements of this gas suggest that it is slowly cooling and settling into the potential well of the galaxy at a rate ranging from $\sim 1 M_\odot \text{ yr}^{-1}$ for isolated ellipticals^{8,11,12} to $\sim 1,000 M_\odot \text{ yr}^{-1}$ for the outer regions of central ellipticals in rich clusters^{13,14}. ($M_\odot$ is the mass of the Sun.) Cooling flows are strongly thermally unstable to the formation of cool clumps, and it has been suggested that the formation of low-mass stars continuously removes mass from these flows at all radii^{15,16}. Indeed, there are observational indications that the mass flux declines steadily towards the centre^{13,17}. In the limiting case, thermal instabilities could remove enough material at every radius so that the residual inflow of hot gas would remain subsonic, cooling on a timescale

comparable to or longer than the free-fall time in the galactic potential⁸. Matters are complicated somewhat if the flow passes through a sonic point^{18,19}. In principle, a cold supersonic flow could continue into the nucleus with the mass flux conserved; however, it is more likely that inhomogeneities in the flow, leading to shocks, would reheat some of the gas to a temperature comparable to the virial temperature, while most of the gas condenses into cold blobs which follow ballistic trajectories. Even a small amount of angular momentum will deflect these blobs from radial orbits; the high pressure in the ambient hot gas will compress them, reducing their collision cross-sections and making it likely that they, too, will form stars before they coalesce into an accretion disk at the galactic centre. As in the subsonic case, the characteristic cooling time of the hot gas will be comparable to or longer than the free-fall time.

Suppose there is a black hole at the centre of the galaxy, of mass $M_h = 10^9 M_\odot$ (that is, of mass M_9 in units of $10^9 M_\odot$). The gravitational potential of the black hole begins to dominate the dynamics of the hot gas at a radius

$$R_h \approx GM_h m_p / kT \approx 50 M_9 T_7^{-1} \text{ pc} \quad (1)$$

where T_7 is the temperature of the gas in units of 10^7 K. If the cooling time of the hot gas at $R \geq R_h$ is comparable to or longer than the local free-fall time, then the density of hot gas at R_h , $n(R_h)$, satisfies

$$n(R_h) \leq 10 M_9^{-1} T_7^{3.1} \text{ cm}^{-3} \quad (2)$$

where I have used a power-law approximation²⁰ for the cooling rate at $10^5 \text{ K} < T < 3 \times 10^7 \text{ K}$. The Bondi theory of spherical accretion²¹ then gives an upper limit to the black hole accretion rate (first pointed out by Nulsen *et al.*⁸):

$$\dot{M} \leq 2 M_9 T_7^{1.6} M_\odot \text{ yr}^{-1} \quad (3a)$$

$$\dot{m} \leq T_7^{1.6} \quad (3b)$$

Equations (3a) and (3b) are valid whether or not additional cooling occurs at $R < R_h$.

In fact, the hot inflow is likely to become angular-momentum-dominated before it reaches R_h . If the timescale for angular momentum transport reduces the inflow speed by a factor α , then the upper limits given in equations (3a) and (3b) will be reduced by a factor α^2 . As the cooling time is already of the order of the inflow time at $R \geq R_h$ (the definition of a cooling flow), one can show that the flow at $R \leq R_h$ will never be able to cool by line-cooling or bremsstrahlung, provided α does not decrease too rapidly with decreasing R (ref. 4). Under the assumptions made by Rees *et al.*⁴, the inner parts of the flow will form an ion torus if $\dot{m} < 50 \alpha_{\text{in}}^2$, where α_{in} is the value of α in the inner parts of the flow. This condition is satisfied if

$$T_7^{1.6} < 50 (\alpha_{\text{in}} / \alpha)^2 \quad (4)$$

Again, we conjecture that the highly compressed cold clumps will form stars before they are able to coalesce to form a nuclear disk; hence, only the hot gas participates in the angular-momentum-dominated accretion flow.

Equation (4) is probably satisfied in most elliptical galaxies; therefore, most active elliptical galaxies should possess radiatively inefficient ion tori, consistent with observations. As suggested by Sparke and Shu²², the development of large-scale collimated radio jets may be fostered by the presence of a hot interstellar medium in ellipticals, and suppressed by its absence in spirals. I therefore suggest that the large-scale morphology of the galaxy governs both the large-scale radio structure and the small-scale structure of the active nucleus.

Note that the upper limit on $\dot{m}$ (Equation (3b)) depends only on the temperature of the cooling flow in the galactic potential and is independent of the mass of the hole. For a variety of plausible galactic potentials, $T^{1/2}$ would scale with the central stellar velocity dispersion (excluding the cusp surrounding the black hole), which in turn is correlated with the luminosity of

the galaxy according to the Faber–Jackson relation²³. This chain of argument leads to a predicted correlation between $\dot{m}$ and the luminosity of the host galaxy. In particular, equation (4) may be violated for the most luminous ellipticals. The inflow would become a radiatively efficient thin disk or radiation torus, and the resulting objects would be classified as luminous quasars. Besides residing in elliptical galaxies, these ‘elliptical’ quasars might be expected to differ from ‘spiral’ quasars in their radio properties. If we accept the view of Sparke and Shu²², the distant quasars which produce highly collimated luminous jets extending to several hundred kiloparsecs—such as 4C32.69 (refs 24, 25)—should be examples of this second class of quasars. There are additional indications from optical studies that radio-loud quasars tend to be associated with elliptical galaxies^{26,27}, although these studies are far from definitive.

Certain classes of active ellipticals, notably BL Lac objects and N galaxies, might be considered optically active, yet are not excessively luminous. It is not clear whether these can be easily accommodated in the scheme outlined above. For BL Lac objects, a case can be made that we are viewing an ion torus pole-on. Alternatively, catastrophic Compton cooling resulting from pair production can sometimes collapse the inner regions of an ion torus, converting it to a radiatively efficient thin disk³⁰. Finally, emission lines from photoionized gas have been detected in the nuclei of many ‘normal’ radio galaxies, indicating that some ultraviolet or X-ray continuum is produced in these objects as well. This need not imply a high radiative efficiency of the central engine, as ion tori are expected to produce a certain amount of hard radiation. In fact, a modest radiative efficiency in X-rays may reduce the accretion rate below the value estimated above, by heating the inflowing gas^{28,29}.

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- Adams, T. F. *Astrophys. J. Suppl. Ser.* **33**, 19–34 (1977).
- Malkan, M. A., Margon, B. & Chanan, G. A. *Astrophys. J.* **280**, 66–78 (1984).
- Hutchings, J. B., Crampton, D. & Campbell, B. *Astrophys. J.* **280**, 41–50 (1984).
- Rees, M. J., Begelman, M. C., Blandford, R. D. & Phinney, E. S. *Nature* **295**, 17–21 (1982).
- Begelman, M. C. in *Astrophysics of Active Galaxies and Quasi-Stellar Objects* (ed. Miller, J. S.) 411–452 (University Science Books, Mill Valley, 1985).
- Rees, M. J. *A. Rev. Astr. Astrophys.* **22**, 471–506 (1984).
- Pringle, J. E. *A. Rev. Astr. Astrophys.* **19**, 137–162 (1981).
- Nulsen, P. E. J., Stewart, G. C. & Fabian, A. C. *Mon. Not. R. astr. Soc.* **208**, 185–195 (1984).
- Forman, W., Jones, C. & Tucker, W. *Astrophys. J.* **293**, 102–119 (1985).
- Trinchieri, G. & Fabbiano, G. *Astrophys. J.* **296**, 447–457 (1985).
- White, R. E. & Chevalier, R. A. *Astrophys. J.* **280**, 561–568 (1984).
- Sarazin, C. L. *Proc. Green Bank Workshop Gaseous Galactic Haloes* (National Radio Astronomy Observatory, in the press).
- Fabian, A. C., Nulsen, P. E. J. & Canizares, C. R. *Nature* **310**, 733–740 (1984).
- Sarazin, C. L. *Rev. mod. Phys.* **58**, 1–115 (1986).
- Fabian, A. C., Nulsen, P. E. J. & Canizares, C. R. *Mon. Not. R. astr. Soc.* **201**, 933–938 (1982).
- Sarazin, C. L. & O’Connell, R. W. *Astrophys. J.* **268**, 552–560 (1983).
- Fabian, A. C., Arnaud, K. A. & Thomas, P. A. *Proc. IAU Symp.* **117** (in the press).
- White, R. E. & Sarazin, C. L. *Astrophys. J. Suppl. Ser.* (submitted).
- White, R. E. & Sarazin, C. L. *Astrophys. J. Suppl. Ser.* (submitted).
- McKee, C. F. & Cowie, L. L. *Astrophys. J.* **215**, 213–225 (1977).
- Bondi, H. *Mon. Not. R. astr. Soc.* **112**, 195–204 (1952).
- Sparke, L. S. & Shu, F. H. *Astrophys. J.* **241**, L65–L68 (1980).
- Faber, S. M. & Jackson, R. E. *Astrophys. J.* **204**, 668–683 (1976).
- Potash, R. A. & Wardle, J. F. C. *Astrophys. J.* **239**, 42–49 (1980).
- Owen, F. N. & Puschell, J. J. *Astr. J.* **89**, 932–957 (1984).
- Malkan, M. A. *Astrophys. J.* **287**, 555–565 (1984).
- Gehren, T., Fried, J., Wehinger, P. A. & Wyckoff, S. *Astrophys. J.* **278**, 11–27 (1984).
- Begelman, M. C., McKee, C. F. & Shields, G. A. *Astrophys. J.* **271**, 70–88 (1983).
- Begelman, M. C. *Astrophys. J.* **297**, 492–506 (1985).
- Begelman, M. C., Sikora, M. & Rees, M. J. *Astrophys. J.* (submitted).

Secular change in solar activity derived from ancient varves and the sunspot index

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The Hale solar period, $\tau_{H(1)} \approx 22$ yr, and its second harmonic, $\tau_{H(2)} \approx 11$ yr, which characterize the sunspot index and are thus hydromagnetic, are imprinted in certain varved rocks of Archaean¹ to late Precambrian^{2,3} age, but the varves record climate and thus are sensitive to solar insolation^{2,3}. We report here apparent collective behaviour of the two kinds of data. The 22-yr/11-yr amplitude spectral density (ASD) ratio, β_i ($i = 1$) of the sunspot index (which is a measure of solar-cycle asymmetry), plotted together with β_i s from three ancient varve thickness sequences against the age for each β_i , results in a remarkably close fit to a decreasing exponential with time constant $\tau \approx 2$ Gyr. These varves and the sunspot index appear to link solar luminosity and the solar field, implying that a common forcing mechanism affects luminosity and the dynamo periods through the common decay time, τ .

Varve data comprise a sequence of thickness values of sediments or chemical layers indexed by integers hypothesized to correspond to solar years. The observation of ~11- and ~22-‘year’ periodicities lends strength to the hypothesis. (The lack of exact correspondence for all the data of this report is discussed below.) Data of the quality of the Elatina sequence² have been reported nowhere else; the Hamersely group¹ has much more material but has not been fully surveyed for data pertaining to the solar problem. In view of the paucity and special qualities of these data, it cannot be guaranteed that further ‘finds’ will not alter the interpretation given here; nevertheless, we think it

important to report these very tentative findings for their potential role in solar physics. The four sources of data used to determine the β_i s and τ were: (1) the Weeli Wooli (Hamersely) banded iron formations¹ (2.5 Gyr BP); (2) the Wolongorang (McArthur basin) dolomitic varves⁴ (1.75 Gyr BP); (3) the Elatina sedimentary varves² (0.67 Gyr BP); and (4) the present-day sunspot index, representing a significant spread in ages. Errors in ages are uncertain, but are in all cases much smaller than the confidence spreads shown in Fig. 2. For Weeli Wooli (thin-section photographic transparency) and Wolongorang (thin section) the procedure for determining varve thicknesses was that used previously⁵ for the light bands in the Elatina sequence, namely, measurement of thickness using a long stage-micrometer with an optical shaft-encoder fed to a small computer for recording.

For computing spectra we generally used the preferred digital Fourier transform (DFT), supported on occasion by a maximum entropy method (MEM). The sunspot index spectrum (computed by DFT) is shown in Fig. 1a, b. In a the plot is linear-linear for clarity, whereas b is log-linear (and filtered) to enhance the small response near 22 yr. We used the prior knowledge of these periods to aid identification of the region in frequency space (unsmoothed for maximum resolution) where the Hale line and its second harmonic were expected. For Elatina those periods were previously determined to be ~12 and 24 yr (ref. 5), rather than the modern 11 and 22 yr from the sunspot index. For the more ancient Wolongorang varves the shift was greater, corresponding to periods of ~14.8 and 28.8 yr, respectively. (A correspondingly greater shift for Weeli Wooli is not apparent, but it does have a 23.3-yr period¹.) The ‘solar’ lines were further certified as the strongest present, and also as satisfying closely a 2:1 ratio of periods. For the final ASD estimates used to calculate β_i s, a simple 5-point boxcar averaging filter was used to smooth the spectra.

As the DFT estimates follow a χ^2 distribution, the β_i s (being a ratio) follow an F distribution. Our next point, regarding degrees of freedom, is crucial to the estimation of the confidence

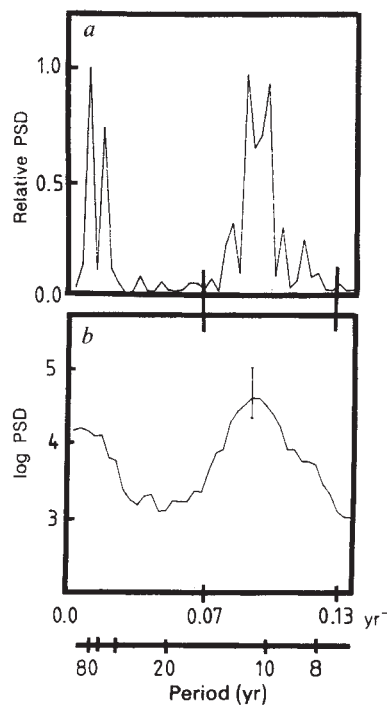


Fig. 1 Digital Fourier power spectral density (PSD) of the Wolf yearly averaged sunspot index. *a*, Unsmoothed 2-DOF relative amplitude, plotted against frequency (upper x-axis) and period; linear-linear plot. Plot *b* (log-linear) emphasizes minor 22-yr power by using a logarithmic ordinate. Error bar, 90% χ^2 confidence limit. This present-epoch case shows $\beta_1 \approx 0.1$.

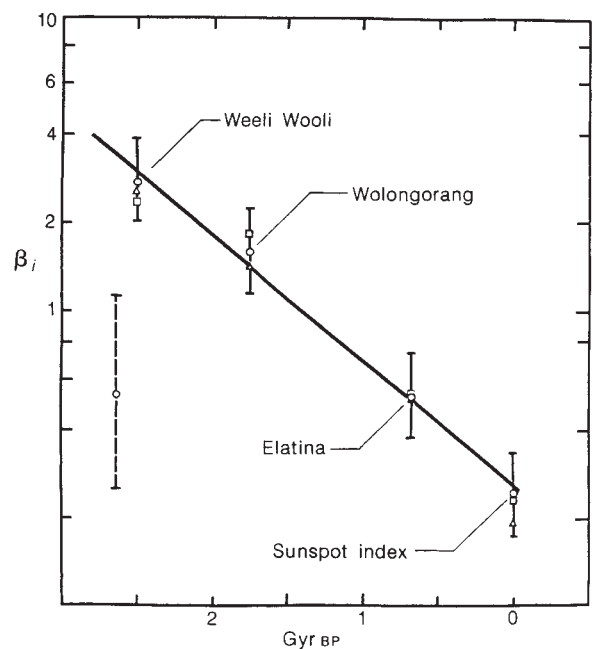


Fig. 2 Least-squares fit of the four values of β_i . The vertical errors are estimated from the F distribution function with 40 DOF. (As noted in the text, in this range of large DOF, F varies little.) Horizontal errors from geochronology and stratigraphy are unimportant compared with those of the β_i s and are not shown. $\circ$, 5-datum filter; $\square$, 3-datum filter; $\triangle$, unfiltered. Solid line, semi-log fit $y = a \exp(-bt)$ with $b = 2 \text{ Gyr}^{-1}$; dashed line, confidence estimate for a single value of β with 10 DOF; that is, the worst possible case (independent or uncorrelated β_s).

intervals. Assuming that all four β_i s are independent, the filtering yields 10 degrees of freedom (DOF) per DFT datum and narrows the χ^2 confidence range considerably. But these data must be and are obviously correlated. The optimistic limit would be ~ 40 – 80 DOF per datum. For the 80% confidence level of the final F distributions, the sensitivity of the confidence range to changes in the number of DOF near 40 is small⁶, and the 80% ranges in Fig. 2 are reasonable. (In the indexing of β_i , i increases with age.)

Fig. 2 shows the least-squares fit of the β_i s to an exponential function. The fit is computed assuming equal relative weighting, as all confidence levels are equal. Although significant deficiencies exist in the Wologorang data, it is remarkable that β_3 lies almost exactly on the least-squares line. (As it is only the ratios of ASDs which are required, common-mode sources of error, which are independent of frequency, are unimportant.) The secular trend in the β_i s and the time constant τ are most readily explained as being due to a relict magnetic field⁷—but only if it is accepted that somehow the varves are proxy field detectors and therefore equivalent to the sunspot index in detecting the hydromagnetic asymmetry found in that index.

The decay of an axially symmetric eddy current for an isotropic sphere of radius R , with homogeneous conductivity σ , has a time constant τ_s , given by

$$\tau_s = \mu_0 \sigma R^2 / \pi^2 s^2 \text{ (MKS)} \quad (1)$$

where $\mu_0 = 4\pi \times 10^{-7} \text{ H m}^{-1}$ is the permeability of free space and s is the multipole order. It is reasonable to take $s=1$, for a relict field must be of odd order to explain the sunspot sequence asymmetry. As τ_s varies inversely with s^2 , it seems unlikely that any higher-order multipole from the time of the Sun's formation is still significant. Cowling⁸ computed the value $\tau_s = 5 \text{ Gyr}$ for a relict solar field using a homogeneous sphere of radius equal to half the solar radius and $\sigma = 10^7 \text{ S m}^{-1}$; the value of τ_s found here is in reasonable agreement. Estimation of τ_s is made more complex by the strong variation of conductivity with temperature

(and therefore radius), and because the circulating currents are likely to occur where the conductivity gradient is largest. Cowling's⁸ conductivity varies with solar radius by nearly three orders of magnitude. Considering the paucity of data, we are not prepared to claim that a relict magnetic field must be responsible for the apparent joint 'bolometric'–hydromagnetic behaviour summarized in Fig. 2, but this is the best interpretation of the data available so far.

Although the Wologorang data is of marginal quality, it covers a crucial time period ($\sim 1.75 \text{ Gyr BP}$); the short (54 data sequence had to be subjectively selected from a longer sequence displaying distortion similar to the inclusions seen in the Weeli Wooli formation, possibly due to diagenesis. The dominant spectral peak positions for the Wologorang varves may be artefacts of the short and distorted record, but the lines chosen are the strongest ones of the DFT and adhere closely to the 2:1 rule regarding harmonic periods. As both the Elatina and Wologorang varves exhibit 'solar' lines displaced from the modern values, the periods increasing with age, perhaps something more may be indicated, such as a secular decrease in the convective zone thickness with age, in turn affecting the dynamo period. However, this must be regarded as conjecture until more data become available.

The major issue raised by these data is why luminosity and magnetic activity should appear to be linked, as shown in Fig. 2. The magnetic field is favoured as the basic variable only because it is the only solar parameter, except the core composition itself, that is capable of entering into a secular decay measured in Gyr. A relict field can explain the asymmetry in the sunspot sequence (or equivalently why both 22- and 11-yr periods are present), provided that the sunspot index varies with the energy in the field⁹, that is, with B^2 . The sunspot index $y(t)$ can be predicted by the equation

$$y(t) = [\delta + \beta \cos(\omega_c t)]^2 \\ = \delta^2(1 + \epsilon^2/2) + 2\epsilon^2 \cos(\omega_c t) + (\epsilon^2/2) \cos(2\omega_c t) \quad (2)$$

where β is the Hale-period amplitude in the sunspot index sequence, ω_c the Hale frequency, δ an offset (relict), $\varepsilon = \beta/\delta$, and the ratio of 22- to 11-yr amplitudes is $4\delta/\varepsilon$. A simpler model assumes that $y(t)$ depends only on the magnitude of solar activity, but to explain the asymmetry requires complications beyond the scope of the present work. We cannot distinguish between a steady or secular value of δ and one which oscillates so slowly as to appear constant over the 282 yr of unbroken sunspot index record, but the latter option has difficulty in explaining the very long characteristic time, τ , shown in Fig. 2.

The sunspot asymmetry alone suggests a relatively small value of steady magnetic field strength; the inferred time constant, if attributed to magnetism, is consistent with its being the vestige of a much stronger pre-main-sequence field. Although a field of the magnitude inferred for the present epoch is dynamically unimportant now (except for the angular momentum problem¹⁰), it could explain the appearance of both the 11- and 22-yr activity periods. It also supports the general principle of an early Solar System magnetic field, either a compressed nebular remnant, a consequent of a nebular dynamo, or some combination of these¹¹. Although the results reported here suggest a role for magnetic fields in association with the protosolar condensation, a relict field offers no obvious explanation for the apparently coupled behaviour of insolation and solar activity. Furthermore, even a modest present-epoch relict field is inconsistent with the observed increase of angular velocity with depth

in the deep solar interior^{12,13}, a problem which is much more severe for the ancient Sun. Finally, the discussion here of periodicities bypasses consideration of statistically oriented (for example, strange attractor) models of solar activity, which may require consideration, although there appear to be difficulties with data quality¹⁴.

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Received 30 January; accepted 8 May 1986.

1. Trendall, A. F. *Econ. Geol.* **68**, 1089 (1973).
2. Williams, G. E. *Nature* **291**, 624-628 (1981).
3. Sonett, C. P. & Williams, G. E. *J. geophys. Res.* **90**, 12019-12026 (1985).
4. Jackson, M. J. *Geology* **44**, 301-326 (1985).
5. Williams, G. E. & C. P. Sonett *Nature* **318**, 523-527 (1985).
6. Abramowitz, M. & Stegun, I. A. (eds) *Handbook of Mathematical Functions* (Dover, New York, 1965).
7. Sonett, C. P. *Nature* **306**, 670-673 (1983).
8. Cowling, T. G. *Mon. Not. R. astr. Soc.* **105**, 166-174 (1945).
9. Sonett, C. P. *Geophys. Res. Lett.* **9**, 1313-1316 (1982); **10**, 491 (1983).
10. Rosner, R. and Weiss, N. O. *Nature* **317**, 790-792 (1985).
11. Boyer, D. W. & Levy, E. H. *Astrophys. J.* **277**, 848-861 (1984).
12. Duvall, T. L. Jr & Harvey, J. W. *Nature* **310**, 19-22 (1984).
13. Duvall, T. L. Jr et al. *Nature* **310**, 22-25 (1984).
14. Gudzenko, L. I. & Chertoprud, V. E. *Soviet Astron.-AJ*, **8**, 555 (1965).

Diurnal cycle of tropospheric OH

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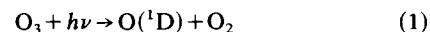
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The hydroxyl free radical, OH, is sunlight's prime agent in maintaining the trace-gas composition of the Earth's troposphere, and at the same time serves as the catalyst responsible for many of the symptoms of urban and regional air pollution. We have used low-pressure fluorescence to measure the ambient concentration of this radical during two continuous 36-hour periods in early summer and late autumn, 1985. The summer and autumn maxima were 3×10^6 and 4×10^5 molecules cm^{-3} (10^{-13} and 2×10^{-14} relative to air), respectively. The observed concentrations lie within the rather large latitude of current estimates based on trace-gas lifetimes or atmospheric photochemical models.

In the global troposphere, reactions with OH are part of a cleansing process, countering natural and man-made inputs. In this contest, interest has centred on methane, the most abundant atmospheric hydrocarbon. Recent observations¹⁻³ imply a persistent imbalance between methane's sources and its removal by OH. Where anthropogenic emissions of other trace gases are concentrated, some of the intermediate products of the OH-catalysed cleansing process are known as urban smog, and some of the final products are known as acid rain. Moreover, because OH controls the atmospheric lifetimes and distributions of most trace gases, secular changes in the tropospheric OH concentration will modify the global thermal radiation budget and the total ozone column.

Direct local observation of tropospheric OH has been notoriously difficult, due to its high reactivity and low concentration. OH measurements have been sporadic, and reported concentrations have varied over a large range, with large measurement uncertainties⁴. Laser-excited fluorescence^{5,6} measurements have suffered from ambient light background, fluorescence of other species, photolytic production of OH by the laser, and

calibration uncertainties. The obstacles to fluorescence determination of OH are significantly lowered by expansion of the air sample to low pressure, in a procedure we have named FAGE (fluorescence assay with gas expansion)⁷. The chief obstacles to previous determinations of OH have been the solar background at the fluorescence wavelength, and production of OH by the laser itself through photolysis of ambient ozone in the sequence:



Here $h\nu$ indicates the exciting laser radiation (which then detects the spurious OH during the same pulse) and $\text{O}(^1\text{D})$ is the first excited state of the oxygen atom. In FAGE, we draw ambient air through an orifice and down a tube to the excitation and detection zone at a pressure of ~ 5 torr. This pressure drop strongly discriminates against step (2) above and against Rayleigh and Mie scattering, the latter particularly a problem in polluted air. The contained environment excludes ambient light. The roughly 100-fold drop in OH concentration associated with the expansion is compensated by an approximately equal increase in the fluorescence yield due to reduced fluorescence quenching at the lower pressure. In addition, reduced quenching results in a longer fluorescence lifetime so that most of the fluorescence waveform is temporally separated from the instantaneously scattered pulse. Finally, the low-pressure transit down the tube to the detection zone allows reagents added at the inlet to react selectively with OH, so that the background signal may be accurately and continuously determined (chemical modulation of the signal).

The FAGE method and instrument have been described elsewhere⁷. To make the measurements reported here, we have substantially improved the sensitivity to ambient OH with a new YAG/dye laser (repetition rate increased from 6 to 30 Hz and 282-nm pulse energy increased from 0.1 to 1 mJ); by replacing the monochromators at $f/4$ (where f is the focal length) by 3-nm (FWHM) multi-layer dielectric filters at $f/1.5$; and by the use of two parallel air-sampling and fluorescence-detection channels, sharing one 0.5-m White cell. A flow of isobutane sufficient to remove 95% of the OH is injected alternately into

the interior of each probe, giving a modulation cycle of 90 s duration. Thus, the signal and the background are measured simultaneously by the pair of probes. Figure 1 is a diagram of the FAGE sampling cell. This sampler was placed 2 m above the roof of our four-story building; the vacuum pump, laser and signal-processing electronics were in the laboratory immediately below.

Our initial goal in developing this technique has been to determine the diurnal concentration profile of OH in tropospheric air, to verify the behaviour projected by atmospheric models. Figure 2 shows the OH concentration observed during a 36-h experiment in Portland, Oregon, on 17–19 June 1985. The darker points are a 1-h moving average; the lighter points are at ± 2 standard deviations, as dictated by the photon counting statistics of the total signal and background. (This uncertainty is twice the square root of the sum of the photon counts from the two probes.) Averaging of the raw data is necessary to reduce noise—a 1-h average results in negligible distortion of the true curve.

The concentration scale in Fig. 2a is determined by the FAGE instrument's OH response, which was measured before and after the experiment, over the range of (intended) measurements. Full calibration of the response (Fig. 3) was carried out by surrounding the sampling probes with an ultraviolet-transparent teflon-film bag, into which flowed a continuous air stream containing trace levels of nitric oxide (NO) and hydrocarbon (mesitylene). The bag was irradiated continuously with ultraviolet fluorescent lamps, and the hydrocarbon, NO and ozone concentrations were measured at the bag inlet and in the interior. A fan ensured that the bag operated as a well-mixed reactor with a residence time t_{res} given by the ratio of its volume V to the total air flow rate F . The input flow was greater than that required by the NO, ozone, hydrocarbon and FAGE measurements, and excess air escaped from the bag through an outlet to the atmosphere. Once steady state has been achieved (as evidenced by unchanging concentrations), mass balance of the hydrocarbon (HC, which reacts only with OH) requires that $F[\text{HC}]_0 - k_{\text{OH+HC}}[\text{OH}] \times [\text{HC}]V - F[\text{HC}] = 0$, where the subscript zero denotes the inlet concentration. Substituting for the bag residence time gives

$$[\text{OH}] = \frac{1}{k_{\text{OH+HC}}t_{\text{res}}} \left(\frac{[\text{HC}]_0}{[\text{HC}]} - 1 \right) \quad (3)$$

For mesitylene, $k_{\text{OH+HC}} = 6 \times 10^{-11} \text{ molecule}^{-1} \text{ cm}^3 \text{ s}^{-1}$ (ref. 8).

The residence time was obtained from the response of [NO] or [HC] to a step change in $[\text{NO}]_0$ or $[\text{HC}]_0$ in the dark. The inlet-to-outlet ratio $[\text{HC}]_0/[\text{HC}]$ was determined by gas chromatography with a variety of lamp intensities and inflow rates of NO, thereby producing a wide range of OH concentrations. This also produced values of the ratio $[\text{NO}]/[\text{O}_3]$ representative of the entire span of NO photo-oxidation in 'classical' photochemical air pollution^{9–11}. This calibrator represents an eulerian approach to each point in the NO photo-oxidation sequence, in contrast to our previous calibration⁷, which employed a lagrangian sweep through the whole sequence. Both give similar results, and in our previous calibration the decay of two separate hydrocarbon species was monitored, each one giving the same value for the OH concentration.

The rather large scatter of the points in Fig. 3 is due in part to the fact that they were obtained over the course of about a week, with variations in laser energy and alignment with roof-top optics un-normalized. The central line in Fig. 3 is the linear-least-squares fit of the measured (hydrocarbon decay) OH concentrations to the FAGE signal.

The precision of the net OH signals in Fig. 2a is equivalent to an error in OH concentration of $\pm 4 \times 10^5 \text{ cm}^{-3}$ at 2 standard deviations (95% confidence, 1-h average). The accuracy of the concentrations in Fig. 2a is limited by the uncertainties in slope and intercept of the calibration in Fig. 3. The intercept uncer-

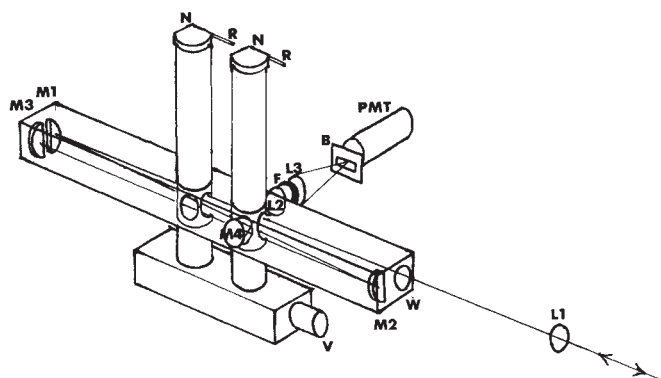


Fig. 1 Diagram of 2-channel FAGE sampler. N, nozzle, 1 mm i.d.; R, reagent/carrier inlet; V, vacuum connection; W, entrance/exit window for laser beam; L1, positive lens focused near W for enlarged beam at sample zone; M1, M2, M3, White-cell mirrors for 18 laser passes through sample; M4, concave mirror; L2, $f/1.5$ lens; F, filter, 4 nm FWHM at 309 nm; L3, $f/2$ lens; B, image mask; PMT, photomultiplier. For clarity, only one of the two detection trains is shown.

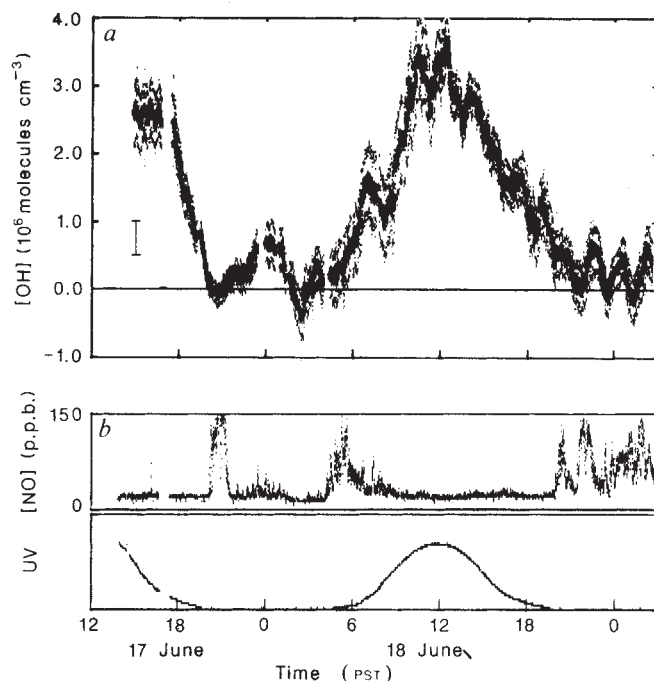


Fig. 2 a, Ambient OH concentration during 17–19 June 1985. The darker points represent a 1-h moving average; the lighter points are at ± 2 standard deviations, based on the photon-counting statistics of the signal and background. The vertical error bar represents the 1σ calibration uncertainty. b, Concurrent measurements of NO concentration (parts per 10^9) and relative ultraviolet intensity (UV) (250–400 nm).

tainty is an inescapable feature of all analytical methods. In Fig. 3, the upper and lower lines are drawn from the 1σ intercept uncertainties using the 1σ slope uncertainties. The vertical displacement of these lines gives the 1σ calibration uncertainty, which is $\sim 5 \times 10^5 \text{ cm}^{-3}$ for the concentration range occupied by the ambient data of Fig. 2. The total photon-counting uncertainty in the calibration is a function of the broad-band fluorescence level of the sampled air. This uncertainty (defined above) is shown as a horizontal error bar and is smaller than those in the hydrocarbon determination in this calibration, which are shown as vertical bars, based on error propagation through equation (3) and using a 2% uncertainty in the determination of the chromatographic peak heights. This calibration uncertainty can be improved in future measurements.

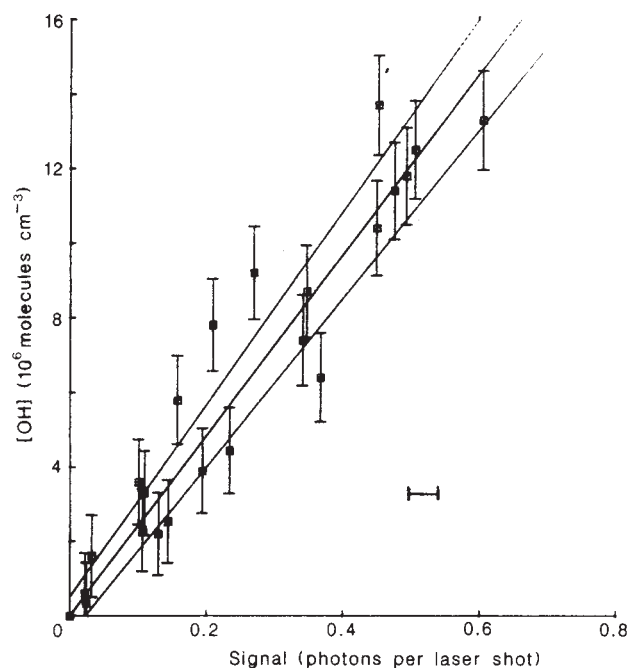


Fig. 3 FAGE calibration using OH-catalysed hydrocarbon consumption in a continuous-flow, ultraviolet-irradiated, Teflon-film bag. Vertical error bars are due to uncertainties in the measurement of hydrocarbon consumption. The horizontal error bar represents the (2σ) photon-counting statistics of the background and signal.

The detailed chemical composition of the air at our urban site is unknown, and its complexity precludes rigorous comparison with tropospheric chemical models. Nevertheless, the diurnal cycle exhibited on the relatively unpolluted day of 18 June (near the summer solstice) is similar to that predicted by clean-air photochemical models at ground level, 45° N latitude. Hydroxyl concentrations in clean air vary largely in response to diurnal variation in ultraviolet light intensity (Fig. 2b). Crutzen and Gidel¹² have calculated a July daytime average [OH] of $2 \times 10^6 \text{ cm}^{-3}$; Logan *et al.*¹³ predicted a diurnal cycle with a peak of $\sim 1.5 \times 10^6 \text{ cm}^{-3}$ at the lower light intensities and water vapour concentrations of the equinox. We used the mechanism and rate constants of ref. 13, with appropriate changes in ultraviolet light intensity and water vapour concentration, to find a maximum clean-air [OH] of $6 \times 10^6 \text{ cm}^{-3}$ at summer solstice. Inclusion of our measured concentrations of NO, O₃, and H₂O reduces the calculated maximum OH concentration to $2 \times 10^6 \text{ cm}^{-3}$. No attempt is made here to include the non-methane hydrocarbon chemistry, which may alter the model predictions further. Graedel *et al.*¹⁴ predicted a peak-summer [OH] of $1.7 \times 10^6 \text{ cm}^{-3}$ in an urban-air modelling study, and Calvert¹⁵ derived a value of $\sim 2 \times 10^6 \text{ cm}^{-3}$ in an analysis of hydrocarbon consumption in the summer Los Angeles atmosphere. Our results are not inconsistent with global [OH] averages and distributions derived from the known sources and atmospheric distributions of CH₃CCl₃ (ref. 16) or ¹⁴CO (ref. 17).

Hydroxyl signals were detected during both nights, but were higher on the first. In contrast, [NO] was much higher on the second night (Fig. 2b). Although daytime photodissociation processes are thought to be the prime source of OH in both clean and polluted air, there are several mechanisms which could account for the presence of OH at night: (1) HO₂ is predicted to persist through the night in clean air at levels $> 10^6 \text{ molecules cm}^{-3}$ (ref. 13), and mixing of such air with urban air containing NO could generate OH; (2) peroxy nitrates serve as free-radical reservoirs which dissociate to generate OH precursors; (3) NO₃ can react with hydrocarbons or aldehydes to give other radicals. Although these sources may require the presence of NO to reduce precursor radicals (such as HO₂) to OH, NO also pro-

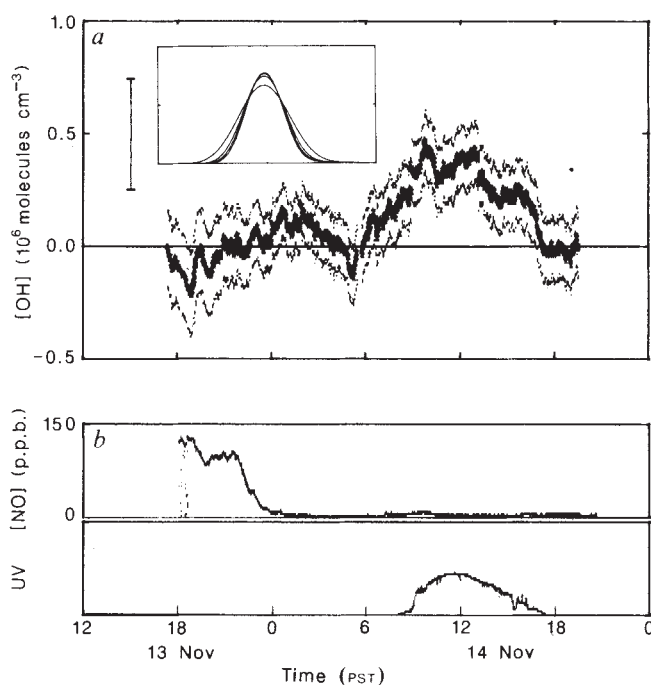


Fig. 4 Ambient [OH] (a) and related variables (b) during 13–14 November 1985. A 4-h averaging time was used for [OH] because of its lower concentration and the higher background relative to the summer measurements; the relative ultraviolet intensity curve cannot be compared on an absolute basis with Fig. 2b; otherwise, as in Fig. 2. The inset shows the effect of several averaging intervals (1, 2, 4 h) on a diurnal [OH] profile generated by numerical integration of a clean-air mechanism.

vides a sink for OH, forming nitrous acid, which is stable in the dark. For a fixed precursor-free-radical source strength, nocturnal [OH] is expected to be inversely related to the NO concentration, consistent with the [OH] signals on the two nights.

Although the night-time OH signals exhibit adequate precision relative to photon-counting uncertainties, their magnitude lies within the calibration uncertainties of Fig. 3; thus, further nocturnal OH measurements are needed.

A second measurement was carried out on 13–14 November 1985, and the results are shown in Fig. 4. Due to lower signal level and higher background, a 4-h moving average is shown here—this will broaden the actual curve, as shown in the figure inset. Here it was necessary to shift the baseline by $2 \times 10^5 \text{ cm}^{-3}$ [OH] to compensate for an apparent negative offset. This offset was not present in the June data and, although of undetermined origin, it may be due to fluorescence of impurities in the modulating reagent. These data show a diurnal pattern similar to that of the summer data, but with a greatly reduced maximum concentration, presumed to be due in large part to reduced light intensity. The maximum concentration is again in agreement with clean-air chemical models, although this agreement may be fortuitous, given the actual composition of the urban air analysed.

The experiments reported here confirm the tropospheric presence of OH and its dependence on light intensity at concentrations in reasonable agreement with tropospheric chemical theory. The experiments do not yet explore the detailed photochemical mechanism that supports OH, which must be understood in order to predict the tropospheric effects of natural cycles and man-made pollution. Besides OH, a key species for this exploration is HO₂, which can also be determined by FAGE⁷. More rigorous comparison of these radical concentrations with clean-air atmospheric models can be achieved by refinement of the calibration of the FAGE instrument, its movement to sites truly representative of the clean troposphere, and the accumulation of data in different seasonal conditions. We

are combining the HO and HO₂ procedures into a single instrument for the initiation of these experiments.

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1. Khalil, M. A. K. & Rasmussen, R. A. *Science* **232**, 56–57 (1986).
2. Rinsland, C. P., Levine J. S. & Miles, T. *Nature* **318**, 245–249 (1985).
3. Stauffer, B., Fischer, G., Neftel, A. & Oeschger, H. *Science* **229**, 1386–1388 (1985).
4. Hewitt, C. N. & Harrison, R. N. *Atmos. Envir.* **19**, 545–554 (1985).
5. Wang, C. C. & Davis, L. I. *Geophys. Res. Lett.* **9**, 98–100 (1981).
6. Davis, D. D., Fischer, S. D. & Rodgers, M. O. *Geophys. Res. Lett.* **9**, 101–104 (1982).
7. Hard, T. M., O'Brien, R. J., Chan, C. Y. & Mehrabzadeh, A. A. *Envir. Sci. Technol.* **18**, 768–777 (1984).
8. Atkinson, R., Darnall, K. R., Lloyd, A. C., Winer, A. M. & Pitts, J. N. Jr *J. Adv. Photochem.* **11**, 375–487 (1979).
9. Perry, R. A., Atkinson, R. & Pitts, J. N. Jr *J. phys. Chem., Ithaca* **81**, 296–304 (1977).
10. Finlayson-Pitts, B. J. & Pitts, J. N. Jr *Angew. Chem.* **14**, 1–14 (1975).
11. Niki, H., Daby, E. E. & Weinstock, H. *Adv. Chem. Ser.* **113**, 16–42 (1972).
12. Crutzen, P. J. & Gidel, L. T. *J. geophys. Res.* **88**, 6641–6661 (1983).
13. Logan, J. A., Prather, M. J., Wofsy, S. C. & McElroy, M. B. *J. geophys. Res.* **86**, 7210–7254 (1981).
14. Graedel, T. E., Farrow, L. A. & Weber, T. A. *Atmos. Envir.* **10**, 1095–1101 (1976).
15. Calvert, J. G. *Envir. Sci. Technol.* **10**, 256–262 (1976).
16. Singh, H. B. *Geophys. Res. Lett.* **4**, 453–456 (1977).
17. Volz, A., Ehhalt, D. H. & Derwent, R. G. *J. geophys. Res.* **86**, 5163–5171 (1981).

Structure determination of α -CrPO₄ from powder synchrotron X-ray data

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Knowledge of the ordered atomic structures of crystalline materials is essential in many areas of science. Single-crystal X-ray diffraction is the technique most often used for both solving and refining crystal structures, but recently, powder diffraction methods have become increasingly important. Many structures have been successfully refined with powder data by using the Rietveld profile method¹ to overcome the problem of overlapping Bragg peaks, but attempts to solve structures *ab initio*, which require a set of well-resolved Bragg intensities, have proved unsuccessful (with a few notable exceptions^{2,3}). However, a new generation of extremely high-resolution powder X-ray⁴ and neutron⁵ diffractometers now allows the *ab initio* determination of crystal structures from powder data (see ref. 6). As part of our study of the structural and magnetic properties of transition-metal phosphates^{7,8}, we present here the structure of α -CrPO₄, solved from powder X-ray data obtained at the Brookhaven National Synchrotron Light Source, and confirmed by a Rietveld analysis of neutron data collected with the diffractometer D1a at the Institut Laue-Langevin, Grenoble.

Two isostructural modifications of CrPO₄ are known⁹. β -CrPO₄ is isostructural with CrVO₄¹⁰, and its atomic and spiral magnetic structure have recently been refined using low-temperature neutron data⁸. Above 1,175 °C, the light-green β phase transforms to the dark-blue α form, considered here. In order to compare the magnetic properties of the two polymorphs in detail, it is necessary to know the structure of α -CrPO₄, but neither this material, nor CrAsO₄¹¹, its only known isotype, has been prepared as single crystals. However, α -CrPO₄ is formed as a highly crystalline powder, making it a suitable candidate for study by high-resolution powder methods.

A polycrystalline sample of α -CrPO₄ was prepared by evaporating a solution containing equimolar quantities of Cr(NO₃)₃·9H₂O (Fisher, certified) and (NH₄)₂HPO₄ (Fisher,

Table 1 Profile and structural parameters from the Rietveld analysis of the second synchrotron data set

Profile parameters

Half-width parameters (0.01°)*

Gaussian $U = 320(15)$, $V = -115(6)$, $W = 12.7(5)$

Lorentzian $X = 4.2(3)$, $Y = 0.46(6)$

Zero-point (0.01°) = -1.50(2)

Cell constants (Å)

$a = 10.40583(11)$, $b = 12.89952(11)$, $c = 6.29933(6)$

Structural parameters[†]

Overall isotropic temperature factor = 0.57(8) Å²

Fractional atomic coordinates in *Imma* (no. 74)

Atom	Symmetry position	x	y	z
Cr(1)	4b	0.5000	0.5000	0.0000
Cr(2)	8g	0.2500	0.3659(3)	0.2500
			0.3650(3)N [‡]	
P(1)	4e	0.5000	0.2500	0.0801(13)
				0.0824(6)N
P(2)	8g	0.2500	0.5740(5)	0.2500
			0.5739(2)N	
O(1)	8i	0.3800(11)	0.2500	0.2275(17)
		0.3778(3)N		0.2267(4)N
O(2)	16j	0.3601(7)	0.4912(6)	0.2155(12)
		0.3613(2)N	0.4903(1)N	0.2146(3)N
O(3)	16j	0.2259(7)	0.6353(6)	0.0576(10)
		0.2234(1)N	0.6367(1)N	0.0553(2)N
O(4)	8h	0.5000	0.3507(8)	-0.0468(16)
			0.3490(2)N	-0.0448(4)N
Bond distances (Å)				
Cr(1)-O(2) (×4)	1.994(8)	P(1)-O(1) (×2)	1.56(2)	
Cr(1)-O(4) (×2)	1.95(1)	P(1)-O(4) (×2)	1.53(2)	
Cr(2)-O(1) (×2)	2.02(1)	P(2)-O(2) (×2)	1.58(1)	
Cr(2)-O(2) (×2)	1.99(1)	P(2)-O(3) (×2)	1.47(1)	
Cr(2)-O(3) (×2)	1.95(1)			

* The two full-width at half maximum (FWHM) functions are: gaussian $\text{FWHM} = (U \tan^2 \theta + V \tan \theta + W)^{1/2}$ and lorentzian $\text{FWHM} = X \tan \theta + Y/\cos \theta$.

[†] Refined coordinates from the neutron experiment at 25 K (marked N) are given for comparison.

certified A.C.S.) until a green, glassy residue remained, and then heating this residue at 1,400 °C for 20 min. Prolonged heating of α -CrPO₄ above 1,175 °C was found to cause decomposition, although the material seems to be stable at ambient temperatures. The composition was confirmed by atomic absorption analysis: observed %Cr = 35.40, %P = 18.27; calculated %Cr = 35.36, %P = 21.08. The X-ray powder pattern agreed with that reported for α -CrPO₄¹², and all the reflections could be indexed on a body-centred orthorhombic cell with $a = 10.405$ Å, $b = 12.898$ Å, $c = 6.297$ Å. We also observed the reflection condition $h,k = 2n$, making *Imma* the highest-symmetry space group possible.

Synchrotron X-ray data were collected on the X13A beam-line at the Brookhaven NSLS. X-rays with a wavelength of 1.323 Å from a perfect Ge(111) crystal monochromator, diffracting in the horizontal plane, impinged upon a flat-plate sample, and the scattering in the vertical plane was measured using a perfect Ge(220) analyser crystal and detector. A full description of the diffractometer and its resolution will be given elsewhere (D. E. Cox, L. P. Carduso, J. B. Hastings & L. W. Finger, in preparation). The sample was mixed with an equal volume of amorphous silica to minimize preferred orientation effects, and the sample tray was oscillated by 1° around the θ -axis to further reduce these effects and to increase the number of crystallites sampled by the beam. Data were collected from 10.50 to 64.50° 2θ at 0.01° intervals, with a count time of 2 s per step. The incident beam intensity was $\sim 5 \times 10^9$ photons s⁻¹, with the storage ring operating at 2.4 GeV and 50 mA.

All of the sharp diffraction peaks observed could be indexed

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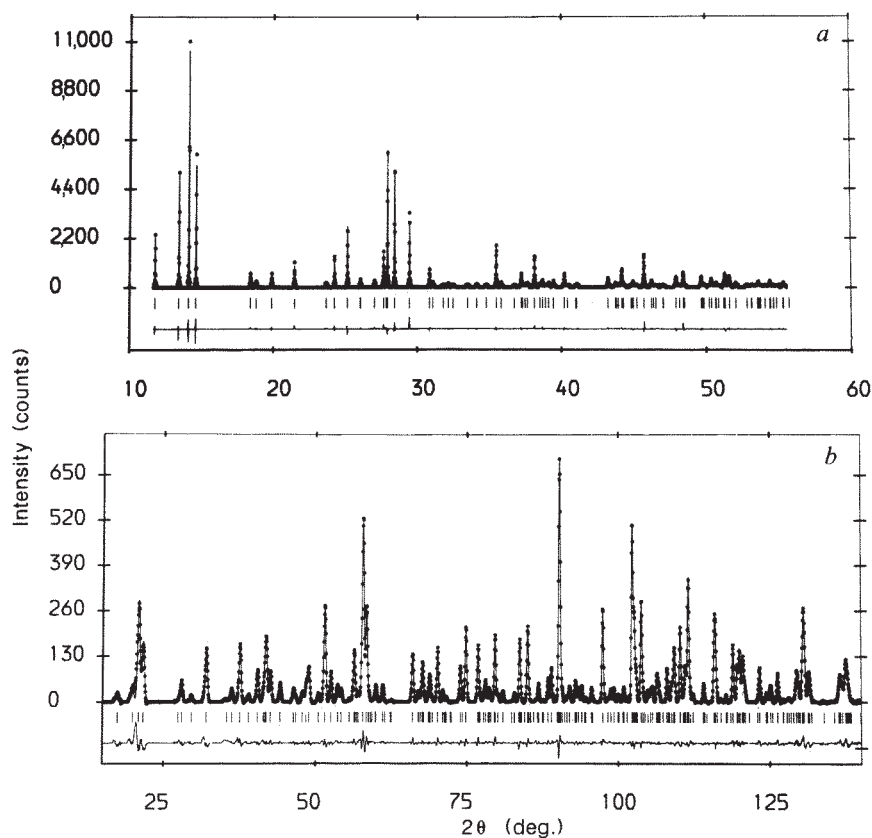


Fig. 1 Observed (points), calculated (bold line), and difference profiles for: *a*, the second synchrotron dataset ($\lambda = 1.323 \text{ \AA}$), and *b*, neutron data collected at 25 K ($\lambda = 1.95 \text{ \AA}$). Reflection positions are marked (short vertical lines).

on the unit cell of $\alpha\text{-CrPO}_4$ in space group *Imma*, and integrated intensities were obtained for 68 well-resolved peaks. After multiplicity and Lorentz corrections, these were used to generate a Patterson map which yielded two heavy-atom positions (Cr(2) and P(2)). These were used to start phasing the data, and the remaining atoms were located using several Fourier maps, bearing in mind geometric considerations. During this process we realized that only 12 formula units were present in the unit cell, instead of 16 as had been proposed¹². The resulting coordinates were used as the starting model for a Rietveld analysis of the entire profile. A modified version of the standard Rietveld/Hewat^{1,13} program was used¹⁴, with peak shapes described by a 'pseudo-Voigt' function¹⁵. This approximates the convolution of gaussian (instrumental resolution) and lorentzian (sample broadening) functions. However, variations in peak shape and poor counting statistics gave a relatively poor fit ($R_{\text{WP}} = 24.1\%$), and did not improve the structural model ($R_{\text{NUC}} = 11.8\%$); R_{WP} and R_{NUC} are defined in reference (1).

To obtain a better refinement of the structure, a second set of synchrotron data was collected from the same $\alpha\text{-CrPO}_4$ /silica mixture, placed in a glass capillary tube (1.0 mm radius) which was rotated around the θ -axis. This reduces preferred orientation effects more effectively than the rocking flat-plate method used above. The region around each Bragg peak position was counted for 4 s per 0.01° step. The longer count time and an increased beam current of 100 mA gave better statistics on the diffraction intensities than those of the first data set. The data were corrected for capillary absorption and the above starting model was refined using the same Rietveld program. A significantly better fit was obtained ($R_{\text{WP}} = 16.9\%$) and the quality of the structure also improved ($R_{\text{NUC}} = 7.0\%$). The results of this refinement are given in Table 1, and the observed, calculated, and difference profiles are shown in Fig. 1*a*.

In addition, during our studies of the low-temperature magnetic properties of $\alpha\text{-CrPO}_4$, a set of neutron data was collected using diffractometer D1a at the ILL, Grenoble. The scattering from a sample of pure $\alpha\text{-CrPO}_4$, held in a vanadium can in a cryostat at 25 K, was measured in the range $6\text{--}136^\circ 2\theta$ in 0.05°

steps at a mean neutron wavelength of $1.95 \text{ \AA}$. A Rietveld analysis of this data set, which contains no magnetic diffraction peaks, gave an excellent fit ($R_{\text{WP}} = 10.4\%$), as shown in Fig. 1*b*, confirming the structure and showing that there is no evidence for the symmetry of the structure being lower than *Imma*. The refined coordinates are given in Table 1 for comparison with the X-ray values; a fuller discussion and more details of these refinements will be presented in a future publication.

The structure of $\alpha\text{-CrPO}_4$ consists of an infinite network of linked CrO_6 octahedra and PO_4 tetrahedra. Pairs of edge-sharing Cr(2)O_6 octahedra, interconnected by P(2)O_4 tetrahedra, form sheets in the *b-c* plane (Fig. 2). These are linked to each other

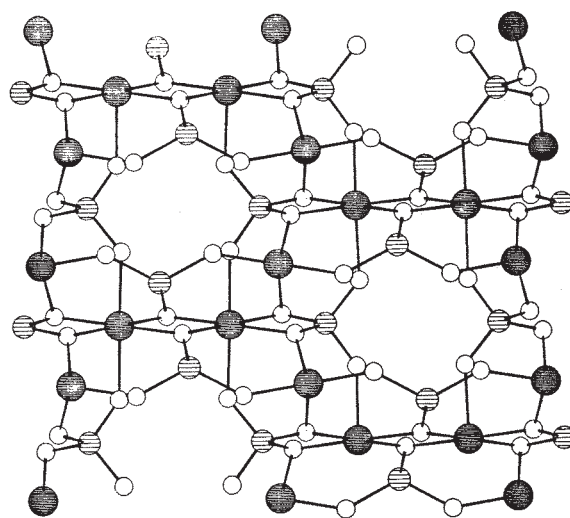


Fig. 2 View of the (100) plane of $\alpha\text{-CrPO}_4$ (*y* horizontal, *z* vertical), showing the coordination of the bidentate phosphate groups to edge-sharing pairs of CrO_6 octahedra (Cr, large shaded circles; P, medium striped circles; O, small open circles).

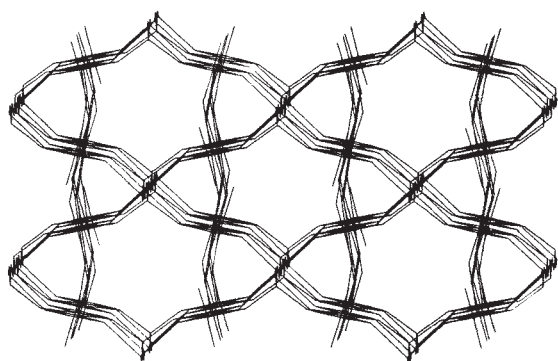


Fig. 3 Stick model of the structure of α -CrPO₄ close to (010), showing the channels parallel to b . x is near-horizontal and z is vertical.

by chains of alternating Cr(1)O₆ octahedra and P(1)O₄ tetrahedra running parallel to b , resulting in the formation of large channels in this direction (Fig. 3). An interesting feature of this structure is the sharing of a common edge between a P(2)O₄ tetrahedron and a Cr(2)O₆ octahedron, both of which are distorted, resulting in a short non-bonding Cr–P distance of 2.68(1) Å and an O–O contact of 2.33(2) Å. The bond distances given in Table 1 and the bond angles (which can be obtained from the authors) are similar to those in β -CrPO₄¹¹ and other chromium phosphates. Although the 3d³ electronic configuration of the Cr³⁺ ion favours regular octahedral coordination, the connectivity of this framework necessarily gives rise to distortions of the polyhedra.

The fact that neither α -CrPO₄ nor CrAsO₄ is thermally stable under the conditions of their formation implies that these compounds are metastable. The persistence of these materials at ambient temperatures may be due to the well-known kinetic

inertness of octahedral Cr³⁺ towards the dissociative ligand exchange that must accompany their decomposition. This also explains why no other trivalent metal phosphate, nor any other ABO₄ compound, has been found to adopt this structure. The cause of this instability is presumably the strained planar four-membered ring resulting from the bidentate phosphate group. The intra-annular angles around Cr(2), P(2) and the two O(2) atoms are 72, 95 and 97° respectively, showing that all are highly distorted from their preferred geometries.

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1. Rietveld, H. M. *J. appl. Crystallogr.* **2**, 65–71 (1969).
2. Berg, J.-E. & Werner, P.-E. *Z. Kristallogr.* **145**, 310–320 (1977).
3. Christensen, A. N., Lehmann, M. S. & Neilsen, M. *Aust. J. Phys.* **38**, 497–505 (1985).
4. Cox, D. E., Hastings, J. B., Thomlinson, W. & Prewitt, C. *Nucl. Instrum. Meth.* **208**, 573–578 (1983).
5. Johnson, M. W. & David, W. I. F. *Rutherford–Appleton Lab. Rep.* No. 85/112 (1985).
6. Cheetham, A. K. *et al. Nature* **320**, 46–48 (1986).
7. Battle, P. D. *et al. J. Phys. C15*, L919–L924 (1982).
8. Attfield, J. P., Battle, P. D. & Cheetham, A. K. *J. Solid St. Chem.* **57**, 357–361 (1985).
9. Shafer, M. W. & Roy, R. *J. Am. chem. Soc.* **78**, 1087–1089 (1956).
10. Frazer, B. C. & Brown, P. J. *Phys. Rev.* **125**, 1283–1291 (1962).
11. Ronis, M. M. C. *r. heb. Séanc. Acad. Sci., Paris* **271C**, 64–66 (1970).
12. Swanson, H. E. *et al. Natn. Bur. Sids. (U.S.)*, **25**, 2, 12 (1963).
13. Hewat, A. W. *U.K. Atom Energy Auth. Res. Gr. Rep. No. RRL73/897* (1973).
14. Cox, D. E. *Acta crystallogr.* **A40**, C369 (1984).
15. Young, R. A. & Wiles, D. B. *J. appl. Crystallogr.* **15**, 430–438 (1982).

Formation of ultra-fine amorphous alloy particles by reduction in aqueous solution

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Amorphous alloys are normally prepared as thin ribbons or films by the liquid quench technique or by vapour deposition. Recently we have shown¹ that ultra-fine amorphous Fe–C alloy particles can be prepared by thermal decomposition of Fe(CO)₅ in an organic liquid; that is, by a chemical reaction. Here we report the preparation of small alloy particles by reduction of metal ions by KBH₄ in aqueous solutions. Mössbauer and X-ray diffraction studies show that the particles are amorphous. The amorphous phase is formed because the chemical reaction takes place below the glass transition temperature and because boron atoms are present in the particles. The method may be used for the large-scale production of ultra-fine amorphous alloy particles, which may have applications in ferrofluids, magnetic memory systems and catalysis.

Reduction of metal ions in aqueous solutions by use of KBH₄ has been described elsewhere^{2,3}. Opegaard *et al.*³ found that reduction of solutions of FeSO₄ or mixtures of FeSO₄ and CoCl₂ by KBH₄ resulted in the formation of small magnetic particles. X-ray diffraction studies of the particles revealed the presence of diffuse lines corresponding to α -iron.

In the present study we have prepared similar material by adding drops of an aqueous solution of FeSO₄ and CoCl₂ with an Fe:Co molar ratio of 7:3 to an excess of 1 M KBH₄. The solutions were mixed by vigorous stirring and the black precipitate was washed in water and then in acetone. Mössbauer spectra were obtained using a constant-acceleration spectrometer with a source of ⁵⁷Co in rhodium. X-ray diffractographs were obtained with a powder diffractometer equipped with a diffracted-beam graphite monochromator. Magnetic measurements were made with a vibrating-sample magnetometer. The samples were studied before and after annealing at 725 K for 2 h in H₂.

Figure 1 shows room-temperature Mössbauer spectra of the sample. Before annealing, the spectrum exhibits very broad lines, indicating a distribution in magnetic hyperfine fields. The isomer shift relative to α -Fe is 0.19 mm s^{−1} and the quadrupole

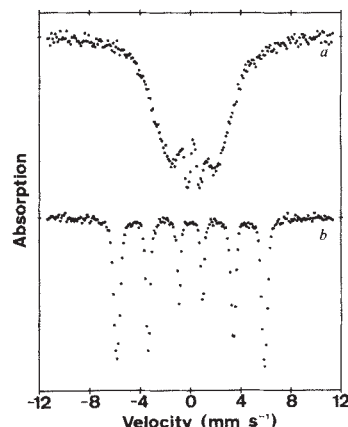


Fig. 1 Mössbauer spectra of the alloy obtained at room temperature: *a*, before annealing; *b*, after annealing at 725 K.

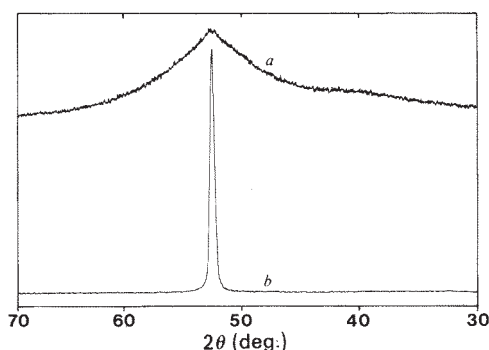


Fig. 2 X-ray diffractograms of the alloy: *a*, before annealing; *b*, after annealing at 725 K. The diffractograms were obtained using Co K α radiation.

shift is negligible. The average magnetic hyperfine field is ~ 18 T, which is considerably smaller than those of crystalline Fe-Co alloys. The magnetic hyperfine field is also smaller than those of amorphous $(\text{Fe}_{1-x}\text{Co}_x)_{80}\text{B}_{20}$ alloys produced by the liquid quench technique⁴, but is similar to those of amorphous Fe-B alloys with higher boron content⁵.

After annealing, the Mössbauer lines are considerably sharper and the average magnetic hyperfine field increases to 36.5 T, whereas the isomer shift decreases to ~ 0.03 mm s⁻¹. These parameters are close to those of a crystalline Fe-Co alloy with an Fe:Co ratio of 7:3 (ref. 6). Thus the Mössbauer results indicate that amorphous Fe-Co-B particles are formed during the preparation and that these particles crystallize during annealing at 725 K.

Further evidence for this conclusion is obtained from the X-ray diffraction studies (Fig. 2). Before annealing, the diffractogram shows only a very broad line, indicating the absence of crystalline phases⁷. After annealing, a sharp diffraction line is observed corresponding to a well-crystallized Fe-Co alloy.

Chemical analysis of the particles showed that the composition of the amorphous alloy is $\text{Fe}_{44}\text{Co}_{19}\text{B}_{37}$; that is, the ratio between iron and cobalt atoms is identical to that in the original aqueous solution. The relatively high boron content explains the low value of the magnetic hyperfine field.

The saturation magnetization of the amorphous $\text{Fe}_{44}\text{Co}_{19}\text{B}_{37}$ alloy is 89 J T⁻¹ kg⁻¹. After annealing it rises to 166 J T⁻¹ kg⁻¹, which is below the value of 240 J T⁻¹ kg⁻¹ for a pure crystalline $\text{Fe}_{70}\text{Co}_{30}$ alloy. The lower value is presumably due to the presence of boron in the alloy or in a separate phase. Infrared spectroscopy of the annealed samples indicated the presence of a boron oxide.

From the Mössbauer and X-ray diffraction spectra it can be concluded that the material formed by reduction of iron and cobalt ions in solution is an amorphous alloy which crystallizes during annealing at 725 K. We have also studied samples prepared from solutions with iron and nickel or with iron alone, and the Mössbauer and X-ray diffraction results for these samples are similar to those of the Fe-Co-B alloy particles. Chemical analysis yielded the compositions $\text{Fe}_{37}\text{Ni}_{28}\text{B}_{34}$ and $\text{Fe}_{62}\text{B}_{38}$, respectively. Electron microscopy of the samples revealed that the particle dimensions are in the range 10–100 nm, depending on the preparation conditions.

Because amorphous alloys are stable only below the glass transition temperature, T_g , a requirement for the formation of amorphous alloy particles by chemical methods is that the chemical reaction takes place below T_g . For pure iron, T_g is far below room temperature; therefore, one or more other elements must be present in order to stabilize the amorphous structure at room temperature. In the present particles, boron atoms from KHB_4 have entered into the alloy particles as a stabilizing element during the chemical reaction.

When amorphous alloys are prepared by the conventional liquid quench technique it is essential that the material is cooled

very rapidly from the melting temperature to a temperature below T_g . This is because the alloys crystallize easily between these temperatures. Therefore, when using this technique, a composition close to the eutectic one facilitates the formation of an amorphous phase. However, when producing amorphous alloys by chemical methods at low temperature the only restriction is that the reaction take place at a temperature below T_g . It is therefore likely that these chemical techniques may allow the formation of amorphous alloys with compositions which cannot easily be produced by the liquid quench technique.

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1. van Wonerghem, J., Mørup, S., Charles, S. W., Wells, S. & Villadsen, J. *Phys. Rev. Lett.* **55**, 410–413 (1985).
2. Schlesinger, H. *et al.* *J. Am. chem. Soc.* **75**, 215–219 (1953).
3. Oppgaard, A. L., Darnell, F. J. & Miller, H. C. *J. appl. Phys.* **32**, 184S–185S (1961).
4. Dey, S., Deppe, P., Rosenberg, M., Luborsky, F. E. & Walter, J. L. *J. appl. Phys.* **52**, 1805–1807 (1981).
5. Nakajimi, T., Nagami, I. & Ino, H. *J. Mater. Sci. Lett.* **5**, 60–62 (1986).
6. Johnson, C. E., Ridout, M. S. & Cranshaw, T. E. *Proc. phys. Soc.* **81**, 1079–1090 (1963).
7. Klug, H. P. & Alexander, L. E. *X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials*, 2nd edn (Wiley, New York, 1974).

Latter-day origin of diamonds of eclogitic paragenesis

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Syngenetic mineral inclusions in diamonds provide the best means of determining their origin. Among such inclusions, the peridotitic paragenesis of olivine, orthopyroxene and chrome-pyroxene garnet is on average far more abundant than the eclogitic paragenesis of pyrope-almandine garnet and omphacitic clinopyroxene^{1–3}. Diamonds of peridotitic paragenesis from the ~ 100 -Myr-old Kimberley and Finsch kimberlites in southern Africa were previously shown to have originated in 150–200-km-thick lithosphere beneath the Kaapvaal craton $\sim 3,300$ Myr ago^{4,5}. At a few localities, such as the Premier kimberlite in southern Africa and the Argyle lamproite in northwestern Australia, diamonds of eclogitic paragenesis predominate^{6–8}, allowing recovery of sufficient material for complementary analysis, the results of which are reported here. Eclogitic garnet and clinopyroxene inclusions in Premier and Argyle diamonds yield Sm–Nd isochron ages of 1,150 and 1,580 Myr respectively, compared with host diatreme emplacement ages^{9,10} of 1,100–1,200 Myr. Eclogitic inclusion ages and precursor isotopic signatures indicate a second genetically distinct origin of diamonds, apparently related in time and space to kimberlite or lamproite magmatism.

The Premier kimberlite is located in the interior of the Archaean Kaapvaal craton and is the only Precambrian diatreme in southern Africa currently exploited for diamonds⁶. It has been difficult to determine the precise age of emplacement because of the altered nature of the kimberlite matrix material, thermal effects of a cross-cutting sill and absence of zircons for U–Pb analysis. Nevertheless, a phlogopite Rb–Sr age of $\sim 1,250$ Myr has been available for some time¹¹, together with a minimum age of 1,100 Myr dictated by the cross-cutting sill¹². Then, following early work by Welke *et al.*¹³, Kramers⁹ obtained a model Pb age of $\sim 1,200$ Myr for presumed sulphide inclusions in Premier diamonds. More recently, two essentially identical Rb–Sr and Sm–Nd ages of $1,180 \pm 30$ Myr have been obtained using diopside and garnet megacrysts^{14,15}.

The Argyle lamproite is located in the Proterozoic Halls Creek mobile zone adjacent to the Kimberley craton in northwestern

Table 1 Rb-Sr and Sm-Nd concentrations* and isotope ratios† for eclogitic garnet and clinopyroxene inclusions in diamonds‡ from the Argyle lamproite and Premier kimberlite

	Wt (mg)	Rb	Sr	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}_i$	Sm	Nd	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}_i$
Argyle§											
d.o.gar	4.61	0.003	15.85	0.0006	0.70588 (2)	0.70587	3.231	5.392	0.3623	0.514205 (16)	0.51045
p.o.gar	1.91	0.009	19.87	0.0013	0.70540 (2)	0.70537	3.220	6.946	0.2803	0.513288 (17)	0.51038
p.g.cpx	0.22	0.381	427.8	0.0026	0.70589 (2)	0.70583	1.084	5.357	0.1224	0.511719 (79)	0.51045
Premier 											
d.o.gar	2.56	0.004	1.74	0.0058	0.70270 (3)	0.70260	0.8545	1.101	0.4696	0.515112 (49)	0.51156
p.o.gar	4.71	0.002	3.00	0.0023	0.70233 (2)	0.70229	0.7463	1.068	0.4226	0.514537 (27)	0.51134
p.g.cpx	1.86	0.167	103.6	0.0047	0.70225 (2)	0.70217	0.5721	2.044	0.1692	0.512840 (63)	0.51156

* Concentrations in p.p.m. ($\mu\text{g per g}$), after correction for minimum applicable blanks: Rb, 3 pg; Sr, 3 pg; Sm, 2 pg; Nd, 2 pg. Estimated analytical uncertainties in concentrations (ignoring small-sample weighing errors) indicated by number of significant digits.

† Present-day $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios normalized to 0.70800 and 0.51264 for standards E & A SrCO_3 and BCR-1, respectively. In-run precision given by errors ($2\sigma_{\text{mean}}$) corresponding to least significant digits. Initial ratios (denoted by subscript i) calculated for Argyle and Premier diamond crystallization ages of 1,580 and 1,150 Myr, respectively, using $\lambda_{\text{Rb}} = 1.42 \times 10^{-11} \text{ yr}^{-1}$ and $\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$.

‡ Selected from -7 + 5 diamond sieve fraction (1.8–2.5 mm diameter) of general mine production.

§ Argyle composites: ~460 deep-orange garnets (d.o.gar) and 190 pale-orange garnets (p.o.gar) (avg. wt 10 μg); ~70 pale-green clinopyroxenes (p.g.cpx) (avg. wt 3 μg).

|| Premier composites: ~175 d.o.gar and 325 p.o.gar (avg. wt 15 μg); ~250 p.g.cpx (avg. wt 7 μg).

Australia, and is the first diamondiferous diatreme in the region to be brought into commercial production¹⁶. A preliminary phlogopite Rb-Sr age of 1,130 Myr for the magmatic phase of the intrusion¹⁰ is consistent with the geochronology of the host country rock succession, which has a maximum age of ~1,200 Myr (refs 16–18).

Premier diamond inclusion mineralogy, abundances and major element compositions are described in detail in ref. 6. The eclogitic assemblage of sulphides, orange garnet, pale-green clinopyroxene and rare kyanite and coesite comprises ~60% of inclusions, with the peridotitic assemblage of sulphides, olivine, orthopyroxene, minor purple garnet and chromite, and rare bright-emerald-green clinopyroxene accounting for the remainder⁶. Argyle diamond inclusion characteristics are outlined in refs 7 and 8. Here the eclogitic assemblage of orange garnet, pale-green clinopyroxene, sulphides, and minor kyanite, rutile and coesite is even more dominant, accounting for up to 90% of the total⁸.

To study the age and origin of these assemblages, the most suitable radiogenic isotope systems are Sm-Nd, Rb-Sr and U-Th-Pb. Although sulphides, the most likely carriers of Pb (ref. 9), appear to be a major inclusion phase at Premier (and to a lesser extent at Argyle), they are difficult to characterize and cannot easily be assigned to either paragenesis^{6,8}. In contrast, the different garnets and clinopyroxenes, the major carrier phases of Nd and Sr, are relatively easily distinguished on the basis of colour, especially after they have been broken out of the host diamonds. This is important because small inclusion size, coupled with Nd concentrations of only a few p.p.m., necessitates the combining of cogenetic inclusions of a given phase⁴. Eclogitic garnets and clinopyroxenes selected for each composite sample in Table 1 were recovered from diamonds bearing monomineralic inclusions, except for scarce Argyle clinopyroxenes, most of which came from diamonds also containing garnets. Touching clinopyroxene and garnet inclusions, which at mantle temperatures would tend to equilibrate continuously with each other by interdiffusion, are rare and such specimens were excluded. Otherwise, Nd and Sr diffusion through intervening diamond is assumed to be negligible⁴. Eclogitic garnets were subdivided on the basis of colour into deep-orange and pale-orange fractions, a distinction which is more clearly developed at Argyle than at Premier. No convincing examples of a pale-orange garnet-clinopyroxene pair were found in available bimineralic-inclusion-bearing diamonds, indicating that pale-orange garnets probably did not coexist with a separate clinopyroxene phase, as also suggested by Sm/Nd partition relationships. Small-sample preparation tech-

niques and mass spectrometric facilities for determination of Sm, Nd, Rb and Sr concentrations and isotope ratios (Table 1) are the same as those used by Richardson *et al.*⁴ except for a new (1985) clean lab (belonging to S.R. Hart) and an improved Sr loading technique with higher ion yield¹⁹.

Nd and Sr concentrations in Argyle inclusions are, on average, five times higher than those in their Premier counterparts, while Sm/Nd ratios are lower by a factor of 1.4 (Table 1). The Sm/Nd ratios, particularly those for garnet (0.5–0.8), are sufficient to produce substantial increases in the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio on a timescale of 1,000 Myr, whereas the Rb/Sr ratios are all extremely low (0.0002–0.002), resulting in barely significant increases in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Thus, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios can be used to verify the cogeneticity of garnet and clinopyroxene inclusions from separate diamonds, which have been combined and paired for the derivation of Sm-Nd isochron age relationships.

Present-day $^{147}\text{Sm}/^{144}\text{Nd}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (Table 1) are plotted on a Sm-Nd isochron diagram in Fig. 1. The Argyle

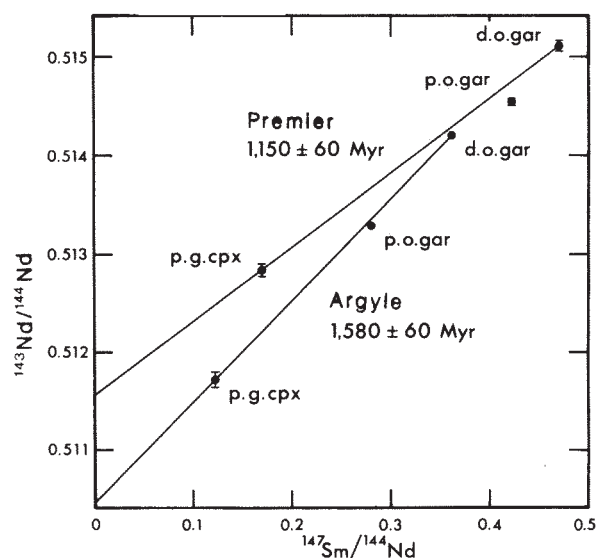


Fig. 1 Sm-Nd isochron diagram for eclogitic garnet and clinopyroxene inclusions in Argyle and Premier diamonds. Measurement errors ($2\sigma_{\text{mean}}$) are indicated except where smaller than the size of plotted points. Ages are for deep-orange garnet-pale-green clinopyroxene pairs, using $\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$. Assigned errors are derived from the maximum and minimum slopes allowed by measurement errors.

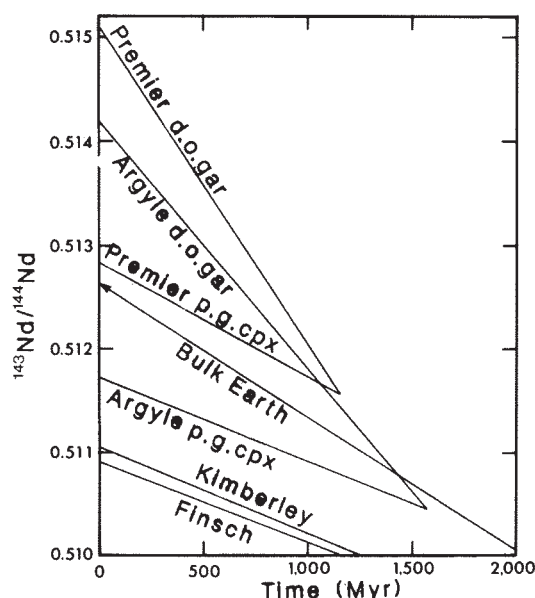


Fig. 2 $^{143}\text{Nd}/^{144}\text{Nd}$ evolution diagram, showing curves for eclogitic garnet and clinopyroxene inclusions in Argyle and Premier diamonds. Curves are constructed from measured $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{147}\text{Sm}/^{144}\text{Nd}$ ratios and terminate at the times of diamond crystallization from Fig. 1. Curves for peridotitic garnet inclusions in Kimberley and Finsch diamonds and the bulk Earth curve are from Richardson *et al.*⁴ and references therein.

deep-orange garnet-clinopyroxene pair defines an age of $1,580 \pm 60$ Myr, with assigned error derived from the maximum and minimum slopes allowed by $2\sigma_{\text{mean}}$ measurement errors (Table 1). The Argyle pale-orange garnet with intermediate Sm/Nd ratio falls below and outside the analytical errors of this two-point isochron. In addition, the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the deep-orange garnet and clinopyroxene are within error of each other (0.7059), while that of the pale-orange garnet is slightly but significantly lower (0.7054). In analogous fashion, the Premier deep-orange garnet-clinopyroxene pair defines an age of $1,150 \pm 60$ Myr, with the pale-orange garnet again having an intermediate Sm/Nd ratio and lying below the two-point isochron. In this case, all three initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are slightly but significantly different (d.o.gar: 0.7026; p.g.cpx: 0.7022; p.o.gar: 0.7023). However, the deep-orange garnet Sr concentration (1.7 p.p.m.) and amount (4 ng) were the lowest measured, and minor sample impurity may have affected the measurement. Thus, the clinopyroxene Sr isotope ratio is considered to be more representative.

The Argyle eclogitic diamond inclusion age of 1,580 Myr is ~ 450 Myr greater than the host lamproite emplacement age¹⁰ of 1,130 Myr, and closer to the $\sim 1,800$ Myr age of stabilization of the surrounding Halls Creek mobile zone¹⁶. Although this indicates that Argyle eclogitic diamonds are xenocrysts in the host lamproite, it does not preclude an original phenocrystal relationship with similar small-volume mantle magmatism ~ 450 Myr earlier and subsequent lithospheric storage. In contrast, the Premier eclogitic diamond inclusion age of $1,150 \pm 60$ Myr is within error of the preferred host kimberlite age^{14,15} of $1,180 \pm 30$ Myr, and in agreement with the sulphide inclusion Pb model age of $\sim 1,200$ Myr obtained by Kramers⁹. There is thus no resolvable time difference between Premier eclogitic diamond crystallization and host kimberlite emplacement. However, the aggregation state of nitrogen in Premier eclogitic diamonds³⁰ requires mantle storage for a finite time interval (~ 1 – 10 Myr at $1,270^\circ\text{C}$, the crystallization temperature⁶; T. Evans and J. W. Harris, personal communication) before removal to the surface.

Further evidence relating to eclogitic diamond origin is pro-

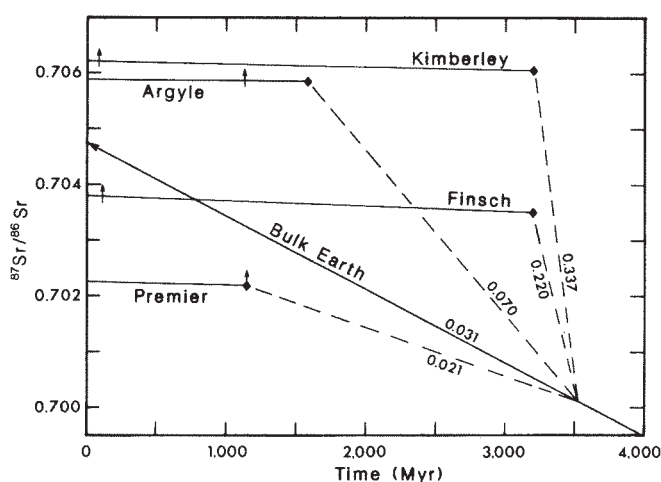


Fig. 3 $^{87}\text{Sr}/^{86}\text{Sr}$ evolution diagram, showing curves for eclogitic garnet and clinopyroxene inclusions in Argyle and Premier diamonds. Curves are constructed from measured $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{87}\text{Rb}/^{86}\text{Sr}$ ratios and terminate at the times of diamond crystallization (diamond symbols) from Fig. 1. Times of host diatreme emplacement are also indicated (vertical arrows). Garnet and clinopyroxene Rb/Sr ratios are so low that the corresponding evolution curves are virtually horizontal. Hypothetical precursor evolution curves (dashed) emanating from the bulk Earth at 3,500 Myr and corresponding time-integrated Rb/Sr ratios are shown for reference. Curves for peridotitic garnet inclusions in Kimberley and Finsch diamonds and the bulk Earth curve are from Richardson *et al.*⁴ and references therein.

vided by the initial Nd and Sr isotopic ratios of the inclusions at the time of their crystallization and encapsulation by diamond (Table 1). These are plotted in Nd and Sr isotope evolution diagrams (Figs 2, 3), for comparison with those of potential precursors. The Premier deep-orange garnet-clinopyroxene pair (and to a lesser extent the pale-orange garnet) has a substantially higher initial Nd isotopic ratio ($\epsilon_{\text{Nd}} = +8$) and lower initial Sr isotopic ratio (0.70217) than those of the bulk Earth at 1,150 Myr. These are the isotopic characteristics of asthenospheric upper mantle such as that now giving rise to the most depleted (low time-integrated Nd/Sm and Rb/Sr) mid-ocean-ridge basalts (MORB). Similar, although less extreme, initial Sr and/or Nd isotopic ratios have previously been measured in Premier clinopyroxene inclusions^{13,9}, carbonatite¹³ and megacryst garnet and diopside¹⁵, although apparently not in kimberlite matrix material²⁰. In addition, the majority of Premier eclogitic-inclusion-bearing diamonds have $\delta^{13}\text{C}$ values of -3 to -6 (ref. 21), consistent with a MORB-type mantle carbon source (see, for example, ref. 22) and within the relatively narrow range of -1 to -8 observed for peridotitic-inclusion-bearing diamonds worldwide²³. In contrast, the Argyle deep-orange garnet-clinopyroxene pair (and similar pale-orange garnet) has initial Nd and Sr isotopic ratios ($\epsilon_{\text{Nd}} = -3$; $^{87}\text{Sr}/^{86}\text{Sr}_i = 0.7059$) respectively lower and higher than those of the bulk Earth at 1,580 Myr (Figs 2, 3). These isotopic characteristics are indicative of a mildly enriched (high time-integrated Nd/Sm and Rb/Sr) component (or mixture of depleted and highly enriched components) akin to that found in old subcontinental lithosphere²⁴ and manifest in young West Kimberley lamproites^{25,26}, although the initial ratios for the host Argyle lamproite itself have yet to be determined. Eclogitic-inclusion-bearing diamonds from Argyle have $\delta^{13}\text{C}$ values of -8 to -16 (E. M. Galimov, personal communication), clearly distinct from those at Premier but within the range of values ($+2$ to -25) for eclogitic-inclusion-bearing diamonds worldwide²³.

The asthenospheric isotopic signature and almost immediate removal to the surface of Premier eclogitic diamonds contrasts with the lithospheric Nd and Sr component, ^{13}C depletion and significant lithospheric storage of their Argyle counterparts.

Evidently, eclogitic-inclusion-bearing diamonds do not have a unique age or precursor but appear to be related in time and space to kimberlite or lamproite magmatism. In addition, these latter-day diamonds of eclogitic paragenesis are distinct in origin from those of the dominant peridotitic paragenesis formed ~2,000 Myr earlier in residual yet highly enriched Archaean lithosphere⁴ (Figs 2, 3). Further study of eclogitic-inclusion-bearing diamonds from Cretaceous eruptives such as Orapa, in the Archaean Limpopo mobile belt²⁷, and Monastery, where garnets with high-pressure phase chemistry have recently been described²⁸, should help to elucidate this distinction and constrain global models of diamond genesis²⁹.

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1. Meyer, H. O. A. & Tsai, H.-M. *Miner. Sci. Engng* **8**, 242-261 (1976).
2. Yefimova, E. S. & Sobolev, N. V. *Dokl. Akad. Nauk SSSR* **237**, 1475-1478 (1977).
3. Hawthorne, J. B., Gurney, J. J. & Harris, J. W. *Ext. Abstr. 12th int. miner. Ass. Meet.* (Novosibirsk, 1978).

4. Richardson, S. H., Gurney, J. J., Erlank, A. J. & Harris, J. W. *Nature* **310**, 198-202 (1984).
5. Boyd, F. R., Gurney, J. J. & Richardson, S. H. *Nature* **315**, 387-389 (1985).
6. Gurney, J. J., Harris, J. W., Rickard, R. S. & Moore, R. O. *Trans. geol. Soc. S. Afr.* (in the press).
7. Hall, A. E. & Smith, C. B. in *Kimberlite Occurrence and Origin: A Basis for Conceptual Models in Exploration* (eds Glover, J. E. & Harris, P. G.) 167-212 (University of Western Australia, Nedlands, 1984).
8. Harris, J. W. & Collins, A. T. *Ind. Diam. Rev.* **45**, 128-130 (1985).
9. Kramers, J. D. *Earth planet. Sci. Lett.* **42**, 58-70 (1979).
10. Skinner, E. M. W., Smith, C. B., Allsopp, H. L., Scott-Smith, B. H. & Dawson, J. B. *Trans. geol. Soc. S. Afr.* (in the press).
11. Barrett, D. R. & Allsopp, H. L. *Ext. Abstr. 1st int. Kimberlite Conf.* 23 (University of Cape Town, 1973).
12. Allsopp, H. L., Burger, A. J. & Van Zyl, C. *Earth planet. Sci. Lett.* **3**, 161-166 (1967).
13. Welke, H. J., Allsopp, H. L. & Harris, J. W. *Nature* **252**, 35-37 (1974).
14. Smith, C. B. thesis, Univ. Witwatersrand (1983).
15. Jones, R. A. thesis, Univ. Leeds (1984).
16. Atkinson, W. J., Hughes, F. E. & Smith, C. B. in *Kimberlites I: Kimberlites and Related Rocks* (ed. Kornprobst, J.) 195-224 (Elsevier, Amsterdam, 1984).
17. Bofinger, V. M. thesis, Australian National Univ. (1967).
18. Plumb, K. A., Derrick, G. M., Needham, R. S. & Shaw, R. D. in *Precambrian of the Southern Hemisphere* (ed. Hunter, D. R.) 205-307 (Elsevier, Amsterdam, 1981).
19. Birc, J. L. *Chem. Geol.* (in the press).
20. Basu, A. R. & Tatsumoto, M. *Contr. Miner. Petrol.* **75**, 43-54 (1980).
21. Deines, P., Gurney, J. J. & Harris, J. W. *Geochim. cosmochim. Acta* **48**, 325-342 (1984).
22. Des Marais, D. J. & Moore, J. G. *Earth planet. Sci. Lett.* **69**, 43-57 (1984).
23. Galimov, E. M. *Geochem. Int.* **22**, 118-142 (1985).
24. Richardson, S. H., Erlank, A. J. & Hart, S. R. *Earth planet. Sci. Lett.* **75**, 116-128 (1985).
25. McCulloch, M. T., Jaques, A. L., Nelson, D. R. & Lewis, J. D. *Nature* **302**, 400-403 (1983).
26. Fraser, K. J., Hawkesworth, C. J., Erlank, A. J., Mitchell, R. H. & Scott-Smith, B. H. *Earth planet. Sci. Lett.* **76**, 57-70 (1985).
27. Gurney, J. J., Harris, J. W. & Rickard, R. S. in *Kimberlites II: The Mantle and Crust-Mantle Relationships* (ed. Kornprobst, J.) 1-9 (Elsevier, Amsterdam, 1984).
28. Moore, R. O. & Gurney, J. J. *Nature* **318**, 553-555 (1985).
29. Haggerty, S. E. *Nature* **320**, 34-38 (1986).
30. Davies, G. *Nature* **290**, 40-41 (1981).

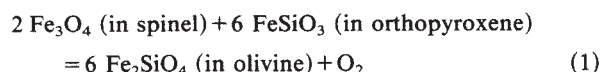
Upper mantle oxygen fugacity recorded by spinel lherzolites

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The oxygen fugacities (f_{O_2} s) recorded by rocks from the Earth's upper mantle have been the subject of much recent study and controversy¹⁻⁷. Discussion has been stimulated by reported differences of several orders of magnitude between measurements made by different methods (Fig. 1). Oxygen fugacity is an important parameter because, together with temperature, pressure and composition, f_{O_2} controls the petrogenesis of mantle-derived magmas, affects the composition and speciation of mantle fluids, and is an initial input into geochemical models of the evolution of the Earth's crust, mantle and hydrosphere. Here we report the determination of the f_{O_2} recorded by upper mantle spinel-lherzolite xenoliths entrained in alkaline magmas. This was done by experimentally calibrating the activity of the Fe_3O_4 (magnetite) component in $MgAl_2O_4$ -rich synthetic spinel and applying these data to calculate thermobarometric f_{O_2} s for the three-phase assemblage olivine-orthopyroxene-spinel. Results for appropriate model xenolith compositions, corrected to 15 kbar total pressure⁸ and plotted in temperature- f_{O_2} space, fall at or above the synthetic quartz-fayalite-magnetite buffer (Fig. 3). In contrast to earlier studies^{2,3}, we conclude that the shallow upper mantle does not retain an f_{O_2} signature of equilibrium with the metallic core, and that gaseous species in the C-H-O system will be dominated by CO_2 and H_2O (refs 9, 10), rather than CH_4 and H_2 .

The f_{O_2} recorded by mantle spinel may be determined from either thermobarometric⁵⁻⁷ or intrinsic¹⁻⁴ measurements. In the thermobarometric method, a heterogeneous equilibrium, such as



is used together with analysed compositions of the relevant mineral phases and experimentally calibrated activity-composition relationships to yield an estimate of the f_{O_2} at constant

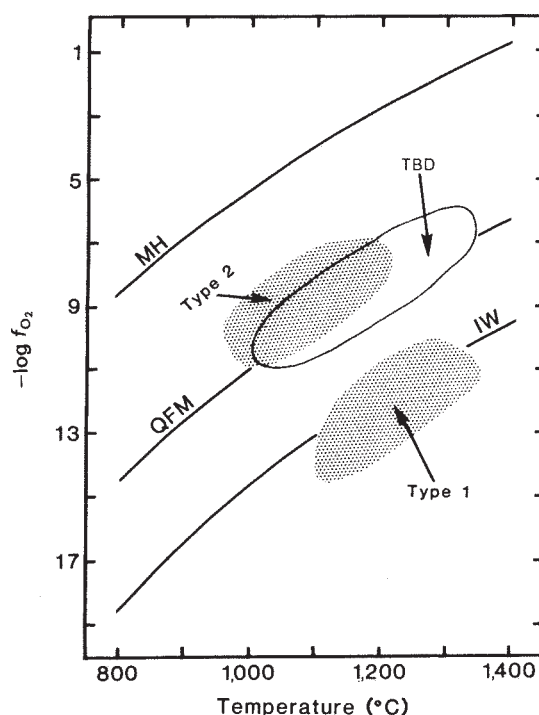


Fig. 1 Compilation of previous estimates of upper mantle f_{O_2} versus temperature at 1 atm total pressure. The stippled regions represent intrinsic measurements of type 2 spinel-lherzolite whole rocks and ilmenite separates⁴ ('Type 2'), and of type 1 spinel separates from spinel-lherzolite xenoliths²⁻⁴ ('Type 1'). The unshaded area ('TBD') represents thermobarometric data from ilmenite-bearing mantle nodules⁵, ilmenite-spinel intergrowths⁶ and terrestrial basalts⁷.

temperature and pressure⁵⁻⁷. In contrast, the intrinsic method is based on determination of single-phase, homogeneous defect equilibria¹¹. It is assumed that the concentration of defects (vacancies in the octahedral sublattice of the spinel phase, for example) is sufficient to buffer the f_{O_2} of a small volume of inert gas at the equilibrium value, which is measured using a zirconia solid electrolyte.

Figure 1 shows previous estimates of upper mantle f_{O_2} together with the standard synthetic Fe-bearing buffers magnetite-haematite (MH), quartz-fayalite-magnetite (QFM) and iron-wüstite (IW) at 1 atm total pressure. The oxygen fugacity estimates fall into two groups. First, thermobarometric estimates⁵⁻⁷ for ilmenite-bearing mantle nodules and megacrysts, spinel-ilmenite intergrowths in nodules found in kimberlite, and Fe-Ti oxides in terrestrial basalts, all group within 1.5 log units of QFM. Second, intrinsic data, collected using a solid zirconia electrolyte technique, show a wide range of apparent f_{O_2} s. Early work on olivine phenocrysts from an oceanic tholeiite pillow¹ and the more recent data²⁻⁴ on spinel separates from type 1 lherzolite xenoliths (Cr-rich suite, Basaltic Volcanism Study Project¹²) are grouped near or below IW. This is up to four orders of magnitude more reducing than the f_{O_2} estimates calculated by thermobarometric methods on the same rocks. Intrinsic measurements of ilmenite separates and type 2 (Cr-poor suite) whole rocks, however, fall at or above QFM⁴. It is unclear why there is such a disparity between the intrinsic f_{O_2} s of type 1 and type 2 spinel lherzolites, as the spinels contain very similar amounts of Fe_3O_4 . Intuitively, the similar mole fractions of Fe_3O_4 imply similar Fe_3O_4 activities and, from equilibrium (1), similar f_{O_2} s. In an effort to resolve the discrepancy, we present an experimentally calibrated data set for dilute magnetite activities on the pseudo-binary spinel join Fe_3O_4 - $MgAl_2O_4$, at 800, 900 and 1,000 °C and 1 atm total pressure. This system approximates closely the compositions of spinels from the type 2 lherzolite suite, and these data, when combined with published experiments on Cr-Al-Fe mixing in spinel¹³⁻¹⁵, may be used to calculate thermobarometric f_{O_2} s for most mantle-derived spinels.

Synthetic spinel solid solutions were prepared initially at 1400–1500 °C in air at 1 atm. Under these conditions, the spinels crystallize in the ternary system $MgAl_2O_4$ - Fe_3O_4 - γ - $Fe_{8/3}O_4$; that is, a small amount of defect spinel (maghemite) is present. Defect spinels were re-equilibrated in a variety of reducing conditions, such as in a 1.0% CO/Ar atmosphere or in a CO/CO₂ gas mix with an f_{O_2} of 10^{-8} at 1,000 °C, and at 1,400 °C in an atmosphere with $f_{O_2} = 10^{-4}$. Ammonium metavanadate titration was used to determine Fe(II)/Fe(III) ratios of selected products. The reducing environments yielded spinels with slightly different defect concentrations; nevertheless, these spinels (between 2 and 20 mol% Fe_3O_4) are essentially stoichiometric within the analytical uncertainty of ~2 mol%. In addition, the spinels were checked for crystallinity and homogeneity by X-ray diffraction, and optical and electron microscopy. Complete details of synthesis conditions, analysis methods, and defect-molar volume relations will be presented elsewhere³¹.

Magnetite activity was measured by mixing haematite with the spinel solid solutions and determining f_{O_2} as controlled by the equilibrium:



We employed an yttria-stabilized-zirconia solid-electrolyte oxygen sensor to measure the shift in f_{O_2} relative to the MH buffer. At constant temperature and known Fe_3O_4 and Fe_2O_3 concentrations in the spinel and orthorhombic phases, respectively, the magnetite activity was given directly by the e.m.f. of the zirconia cell relative to a gas of known f_{O_2} . Al_2O_3 solubility in the haematite phase was modelled using published data¹⁶. At fixed Fe_3O_4 mole fraction, spinels synthesized under oxidized and various reduced conditions yielded the same Fe_3O_4 activity. This implies that the amount of γ - $Fe_{8/3}O_4$ component in the spinel phase must closely approximate the equilibrium concentration at the temperature and f_{O_2} of the experiment. Equilibrium was demonstrated by *in situ* addition or subtraction of oxygen from the sample (coulometric titration) and verification that the e.m.f. was affected by these perturbations. The sample usually approached equilibrium fairly quickly after perturbation, so that the variation in e.m.f. with time became negligible within an

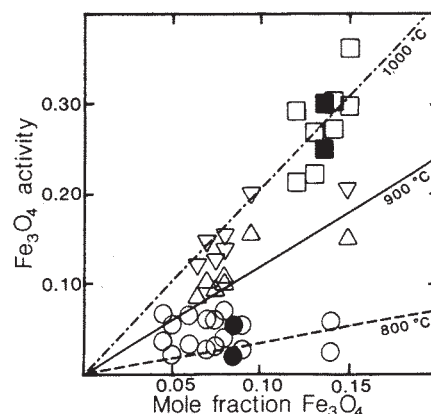


Fig. 2 Experimentally calibrated activity data for the Fe_3O_4 component in the spinel phase, plotted against the mole fraction of Fe_3O_4 component in $MgAl_2O_4$ -rich synthetic spinel at 800, 900 and 1,000 °C (circles, triangles and squares, respectively) and 1 atm total pressure. Open symbols represent approach to equilibrium from high and low f_{O_2} with spinels synthesized in reducing conditions; shaded symbols are for runs with spinels synthesized in air. The size of the symbols reflects the approximate cumulative experimental uncertainty.

hour at 900 °C. X-ray diffractograms were obtained for all run products, to ensure that both phases were present and to determine the final spinel and haematite compositions.

Figure 2 shows the results of our activity measurements, as a plot of the reversed magnetite activities against the final spinel phase composition, determined using X-ray diffraction. We include experiments with spinel solid solutions made in the various reduced conditions as well as two runs with spinels made in air. The size of the symbols represents the approximate cumulative uncertainty for each reversal bracket. At each composition, the pair of symbols represents approach to equilibrium from high (lower symbol) and low (upper symbol) f_{O_2} . Note that our experiments are in the composition and temperature range appropriate for Cr-free mantle spinels. Our observation of increasing magnetite activity with increasing temperature at constant composition is probably due to changes in the equilibrium ordering state of the mixed spinels relative to the pure magnetite standard state¹⁷. This behaviour persists across the entire Fe_3O_4 - $MgAl_2O_4$ join at 900 and 1,000 °C. We have used approximate linear (Henry's law) fits to the dilute magnetite activities (Fig. 2) in order to estimate upper mantle f_{O_2} . The fit lines are calculated from the equation

$$A_{Fe_3O_4} = (\alpha + \beta T) X_{Fe_3O_4} \quad (3)$$

where $X_{Fe_3O_4}$ and $A_{Fe_3O_4}$ are the mole fraction and activity of magnetite in spinel, $\alpha = -6.5 \pm 0.5$, $\beta = 8.5 \pm 0.5 \times 10^{-3}$, and T is temperature in °C.

Upper mantle f_{O_2} recorded by spinel-lherzolite xenoliths, which contain the three-phase assemblage spinel-orthopyroxene-olivine, may now be calculated thermobarometrically from equilibrium (1). Fe_3O_4 activity was taken from equation (3), with Fe_2SiO_4 and $FeSiO_3$ activities and standard-state data from the literature^{18,19,32}. The Mg number ($Mg^{2+}/(Mg^{2+} + Fe^{2+})$) of the silicate phases was fixed at 0.90, and the spinel phase composition was varied between 0.01 and 0.10 mole fraction Fe_3O_4 component. The latter range corresponds to the approximate limits observed by electron microprobe, wet chemistry and Mössbauer spectroscopy for spinel in mantle-derived spinel-lherzolite xenoliths. Figure 2 shows the results of these calculations as a temperature-log f_{O_2} plot at 15 kbar total pressure. Pressure-corrected Fe buffers are also included for reference. The f_{O_2} s shown in Fig. 3 were calculated at 15 kbar pressure using a ternary-spinel molar volume

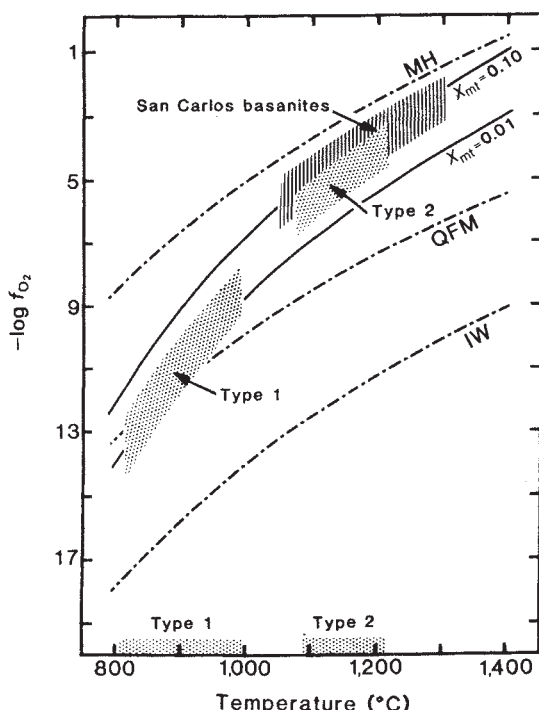


Fig. 3 Thermobarometric spinel data at 15 kbar total pressure. Solid lines are the loci of $(\log f_{O_2}, T)$ data calculated from equilibrium (1) for magnetite mole fractions (X_{mt}) of 0.01 and 0.10 in the Cr-free spinel. Magnetite activity was calculated using equation (3). The stippled regions refer to type 1 and type 2 spinels, with the former case including a correction for Cr substitution. Temperature ranges (shown on the abscissa) are taken from the literature²³. The lined region is the f_{O_2} - T range for the San Carlos basanites²⁵. Note the more reduced f_{O_2} s for type 1 xenoliths, and the considerable overlap in f_{O_2} of type 2 xenoliths with the San Carlos basanites.

model^{8,31} and published data for orthopyroxene²⁰ and olivine²¹, neglecting the small expansivities and compressibilities of the phases. The effect of Cr substitution in the spinel phase was estimated using published phase equilibrium data^{13,14} and the formalism of Sack¹⁵. This approach suggests a slight lowering (<1 log unit) of f_{O_2} relative to the Cr-free system if the spinel contains 25 mol% Cr^{3+} as $FeCr_2O_4$. Preliminary Fe_3O_4 activity experiments on Cr-bearing synthetic spinels confirm that f_{O_2} is not reduced significantly relative to the Cr-free system.

The observed composition and temperature range for natural spinels in spinel lherzolites is shown in Fig. 3 (stippled areas), and gives f_{O_2} s which range from slightly below QFM to approximately two orders of magnitude more oxidized. The type 1 (Cr-rich) suite is certainly more reduced than the type 2 (Cr-poor) suite; however, our estimates for upper-mantle f_{O_2} are all near or above QFM, in general agreement with previous thermobarometric data⁵⁻⁷, and in sharp contrast to the intrinsic data for spinel separates from type 1 xenoliths (Fig. 1)²⁻⁴. Extremely low Fe_3O_4 concentrations ($X_{Fe_3O_4} \approx 10^{-4}$) in the spinel phase would be necessary to stabilize spinel-orthopyroxene-olivine assemblages at an f_{O_2} near IW. However, published analyses²²⁻²⁴ do not in general report such vanishingly small magnetite contents; most spinels from spinel-lherzolite xenoliths contain $X_{Fe_3O_4} \geq 0.01$. The discrepancy between intrinsic and thermobarometric results must be due to errors in some spinel analyses or to carbon interference in some intrinsic measurements; pressure effects on the latter can be discounted^{8,31}. Experiments are now under way to investigate this discrepancy.

Figure 3 also shows the approximate f_{O_2} range for the San Carlos basanites²⁵, corrected to the appropriate temperature and 15 kbar pressure²⁶⁻²⁸. Note the general agreement between the f_{O_2} recorded by type 2 xenoliths and that calculated for the San Carlos basanitic magmas, in which examples of both xenolith suites are entrained. The f_{O_2} data support the conclusion of Frey and Prinz²⁵, based on trace-element abundance patterns of xenoliths from San Carlos and elsewhere, and of Menzies²⁹, based on Sr and Nd isotopic abundances of xenoliths relative to their host magmas, that type 2 xenoliths are cognate, and thus genetically related to the basanites which entrain them, although not necessarily of the same generation. We find no evidence to support the suggestion² that partial melting in the Earth's shallow upper mantle results in a melt-residua system with an f_{O_2} near the IW buffer. This eliminates the need for extensive H_2 loss during magma ascent to explain the observed f_{O_2} s at or above QFM for alkaline and basaltic extrusives⁷.

Our results show that the f_{O_2} accompanying upper mantle processes is not uniform, and may vary from slightly below to approximately two orders of magnitude more oxidized than QFM at 15 kbar total pressure. Our data do not preclude the possibility that low- f_{O_2} zones occur at greater depth or that the mantle was more reduced in Archaean times than at present³⁰. In the spinel-lherzolite facies, however, f_{O_2} s are close to QFM and gaseous species are dominated by CO_2 and H_2O (refs 9, 10), rather than CH_4 and H_2 .

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1. Sato, M. *Mem. geol. Soc. Am.* **135**, 289-307 (1972).
2. Arculus, R. J. & Delano, J. W. *Nature* **288**, 72-74 (1980).
3. Arculus, R. J. & Delano, J. W. *Geochim. cosmochim. Acta* **45**, 899-913 (1981).
4. Arculus, R. J., Dawson, J. B., Mitchell, R. H., Gust, D. A. & Holmes, R. D. *Contr. Miner. Petrol.* **85**, 85-94 (1984).
5. Eggler, D. H. *Geophys. Res. Lett.* **10**, 365-368 (1983).
6. Haggerty, S. E. & Tomkins, L. A. *Nature* **303**, 295-300 (1983).
7. Haggerty, S. E. *Geophys. Res. Lett.* **5**, 443-446 (1978).
8. Mattioli, G. S. & Wood, B. J. *Geol. Soc. Am. Abstr. Progr.* **17**, 655 (1985).
9. French, B. M. *Rev. Geophys.* **4**, 223-253 (1966).
10. Eggler, D. H. & Baker, D. R. in *High-Pressure Research in Geophysics* (eds Akimoto, S. & Manghnani, M.) 237-250 (Center for Academic Publications, Tokyo, 1982).
11. Dieckmann, R. *Ber. Bunsenges. phys. Chem.* **86**, 112-118 (1982).
12. Basaltic Volcanism Study Project *Basaltic Volcanism on the Terrestrial Planets* (Pergamon, New York, 1981).
13. Webb, S. A. C. & Wood, B. J. *Contr. Miner. Petrol.* **92**, 471-480 (1986).
14. Oka, Y., Steinke, P. & Chatterjee, N. D. *Contr. Miner. Petrol.* **87**, 196-204 (1985).

15. Sack, R. O. *Contr. Miner. Petrol.* **79**, 169-186 (1982).
16. Muna, A. *Am. J. Sci.* **256**, 413-422 (1958).
17. Mattioli, G. S. & Wood, B. J. *Proc. 14th Conf. Miner. Soc.* (in the press).
18. Wood, B. J. & Kleppa, O. J. *Geochim. cosmochim. Acta* **45**, 529-534 (1981).
19. Bohlen, S. R., Essene, E. J. & Boettcher, A. L. *Earth planet. Sci. Lett.* **47**, 1-10 (1980).
20. Newton, R. C. & Wood, B. J. *Am. Miner.* **65**, 733-745 (1980).
21. Fisher, G. W. & Medaris, L. G. Jr *Am. Miner.* **54**, 741-753 (1969).
22. Basu, A. R. & MacGregor, I. D. *Geochim. cosmochim. Acta* **39**, 937-945 (1975).
23. Sachtleben, Th. & Seck, H. A. *Contr. Miner. Petrol.* **78**, 157-165 (1981).
24. Fujii, T. & Scarfe, C. M. *Contr. Miner. Petrol.* **80**, 297-306 (1982).
25. Frey, F. A. & Prinz, M. *Earth planet. Sci. Lett.* **38**, 129-176 (1978).
26. Sack, R. O., Carmichael, I. S. E., Rivers, M. & Ghiorso, M. S. *Contr. Miner. Petrol.* **75**, 369-376 (1980).
27. Kilinc, A., Carmichael, I. S. E., Rivers, M. L. & Sack, R. O. *Contr. Miner. Petrol.* **83**, 136-140 (1983).
28. Mo, X., Carmichael, I. S. E., Rivers, M. & Stebbins, J. *Mineralog. Mag.* **45**, 237-245 (1982).
29. Menzies, M. A. in *Continental Basalts and Mantle Xenoliths* (eds Hawkesworth, C. J. & Norry, M. J.) 92-110 (Shiva, Nantwich, 1983).
30. Arculus, R. J. A. *Rev. Earth planet. Sci.* **13**, 75-95 (1985).
31. Mattioli, G. S., Wood, B. J. & Carmichael, I. S. E. *Am. Miner.* (submitted).
32. Myers, J. & Eugster, H. P. *Contr. Miner. Petrol.* **82**, 75-90 (1983).

Crustal detachment during South Atlantic rifting and formation of Tucano–Gabon basin system

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A series of major sedimentary basins of Mesozoic to Tertiary age are associated with the Brazilian continental margin between 8° and 14° S (Fig. 1). This passive continental margin formed when Africa and South America split apart with the initiation of seafloor spreading in the Aptian/Albian³. The offshore basins which border the continental margins on both sides of the Atlantic show evidence of both rifting and crustal attenuation before break-up and thermal subsidence after it. In contrast, a combined stratigraphical and gravity study of the Brazilian onshore basins, which are separated from the offshore basins by a northward-widening strip of Precambrian, suggests that up to 7 km of non-marine sediments were deposited during the rifting stage of the South Atlantic, with no apparent extension of the lower crust beneath these basins and no subsequent thermal subsidence. A mechanism for the linked development of these onshore and offshore basins is proposed here along the lines suggested by Wernicke^{4,5}, in which upper crustal extension in the onshore region is connected to, and balanced against, deeper lithospheric extension beneath the incipient passive margin by intracrustal detachments.

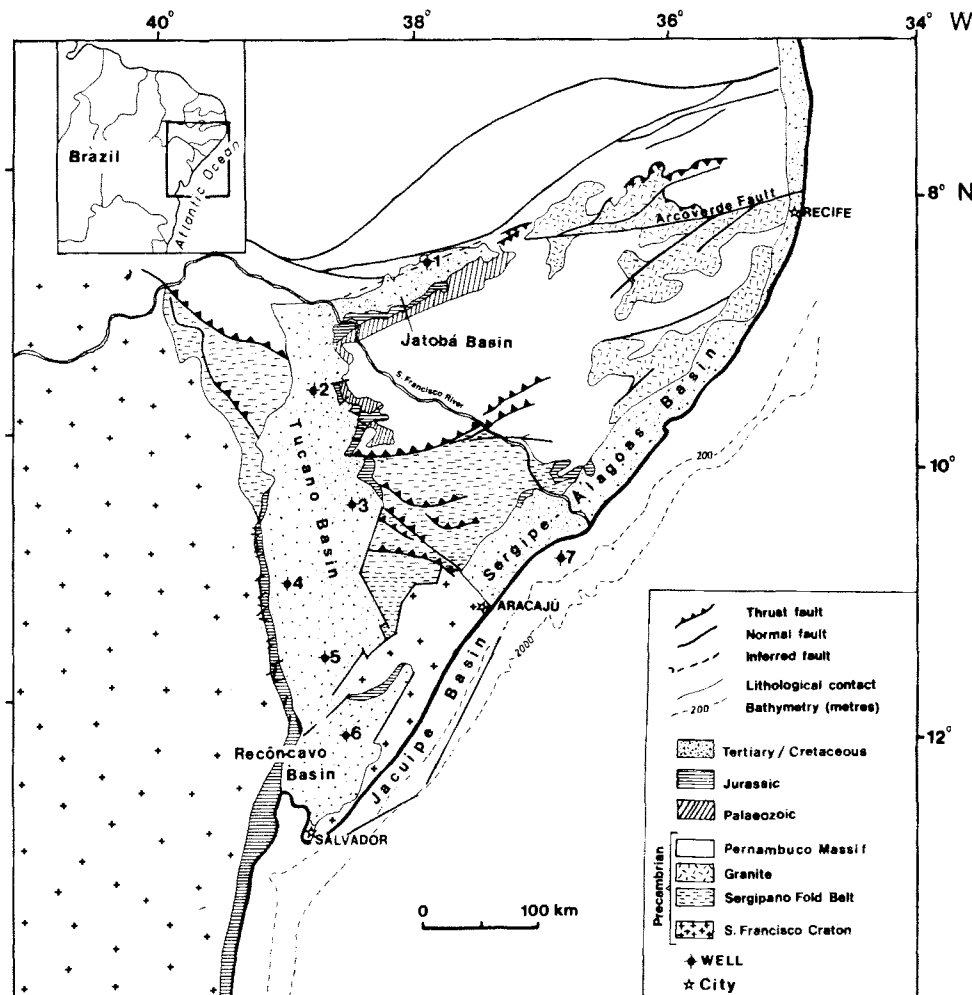
As shown in Fig. 1, the basins adjacent to the Brazilian margin are the coastal Sergipe–Alagoas Basin, and the less well known

offshore Jacuípe Basin which initially adjoined the complementary Gabon Basin on the opposite continental margin. The Brazilian onshore basins extend northward from Recôncavo and include the Tucano and Jatobá 'aborted rift' basins⁶. These are separated from the coast by a triangular Precambrian block.

The Bouguer anomaly map (Fig. 2a) of this section of the Brazilian coast shows that all the basins are characterized by large-amplitude local negative gravity anomalies, indicating thick, low-density sediment infills. The maximum thickness of 7–8 km of sediments in the Tucano Basin produces a local gravity effect of –100 mgal amplitude. Gravity modelling of the Tucano Basin, using sediment densities obtained from density logs of commercial wells, suggests that the observed sediments adequately account for the observed gravity anomaly (Fig. 2b). There is no indication from flanking positive anomalies of significant upwarping of the underlying Moho immediately beneath the basin. In contrast, modelling of the Jacuípe Basin, which has a maximum sediment thickness of ~3–4 km (ref. 8), suggests that considerable crustal thinning and Moho upwarp has occurred beneath this offshore basin (Fig. 2b).

The Tucano Basin thus gives no evidence of local isostatic compensation, suggesting that it has been formed by upper-crustal extension without complementary extension of the underlying rigid lower crust and uppermost mantle. The lack of local Moho topography as determined by the gravity studies suggests that the lithosphere had a large flexural rigidity at the time of basin formation, and that it has remained high since. Assuming that lithospheric flexure can be modelled as a thin elastic plate overlying a weak fluid substratum, an effective elastic plate thickness of 50–80 km produces a compensating gravity effect of only 10 mgal associated with a regional Moho uplift of ~2 km with a wavelength of 1,000 km (Fig. 2c). This

Fig. 1 Geological map of north-eastern Brazil (conical projection), modified from ref. 1 to show the Mesozoic to Tertiary sedimentary basins. The onshore Jatobá, Tucano and Recôncavo basins cut across the NW–SE-trending Sergipano fold belt, which extends into Gabon and Angola as the Ndjolé Series². 1–7, Location of wells used in this study.



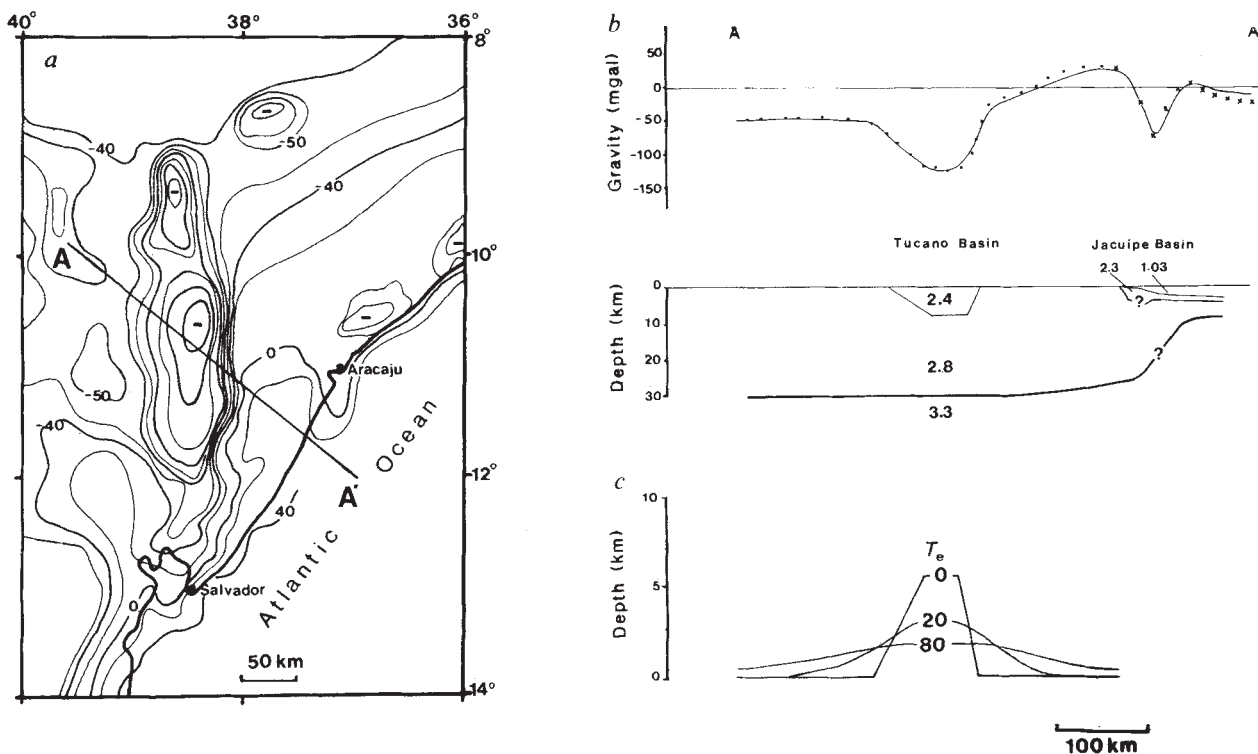


Fig. 2 *a*, Bouguer anomaly map of northeastern Brazil approximately coinciding with Fig. 1, using Bouguer density of 2.67 g cm^{-3} and referenced to IGSN71. Contour interval is 20 mgal. Source: Petrobras and DNP/Brazil. *b*, Interpretation of a gravity anomaly profile across the Tucano and Jacuípe basins (A-A' in *a*), based on the Bouguer anomalies (points) and free-air marine anomalies⁷ (crosses). The curve is the gravity anomaly profile calculated from the crustal model shown below, in which average densities from commercial wells are shown in g cm^{-3} . In the absence of deep seismic data, the depth to the Moho beneath the craton has been assumed to be 30 km. *c*, Upwelling of the Moho produced by the Tucano Basin upper-crustal mass deficiency, plotted as a function of the effective elastic thickness T_e of the lithosphere. Moho topography consistent with gravity observations suggests T_e is between 50 and 80 km.

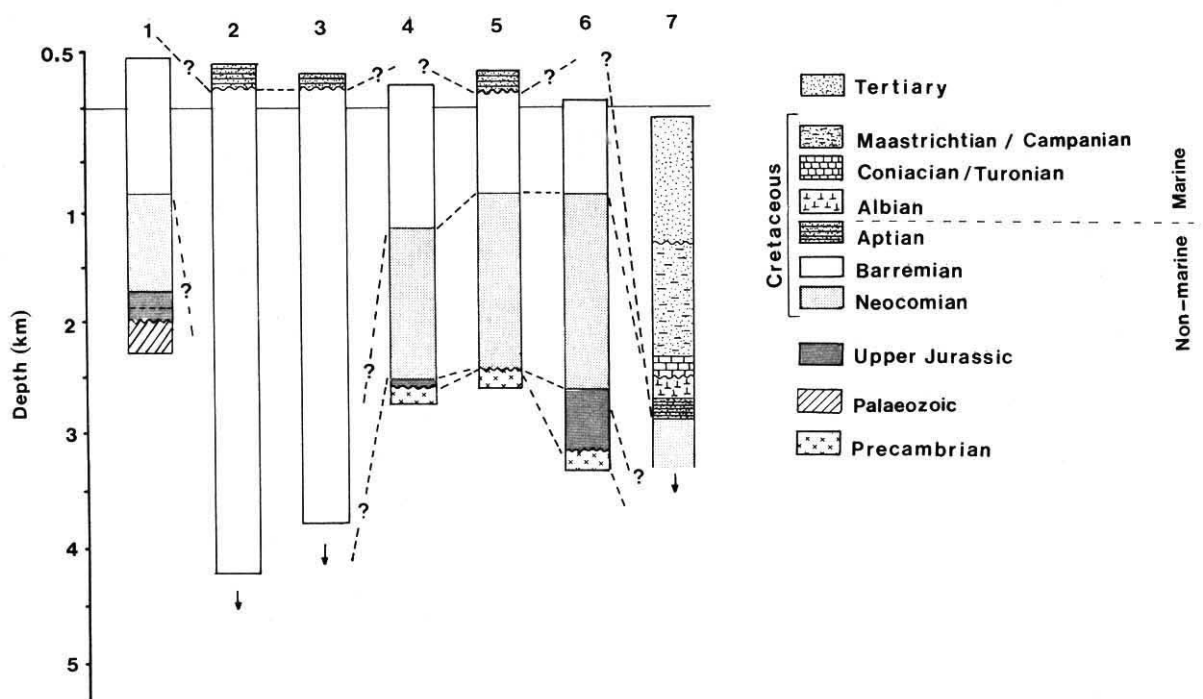


Fig. 3 Simplified stratigraphy of the Jatobá, Tucano, Recôncavo⁹ and Sergipe-Alagoas¹⁰ basins, based on well data provided by Petrobras (Fig. 1). The Upper Jurassic and Neocomian in all basins consist of non-marine shales and sandstones with intercalations of arkose and siltstone. Non-marine sandstones and siltstones of great thickness dominate the onshore basins in the Barremian, and post-rift marine sediments are found only in the Sergipe-Alagoas Basin (well 7).

long-wavelength gravity effect would contribute to the regional anomaly, and the local basin would appear to be uncompensated. Furthermore, the existence of a large rigidity at the time of basin formation implies that the rifting responsible for the Tucano and Jatobá basins was not associated with any significant modification of the thermal structure of the lithosphere immediately below.

Although gravity is useful in defining the present structure of the basins and their isostatic state, it cannot describe their temporal development. However, the stratigraphic evidence obtained mainly from deep wells in the basins is potentially a sensitive indicator of the history of basin subsidence, and thus a further pointer towards the mechanism of formation.

Figure 3 shows the subsidence history and stratigraphy of the Recôncavo, Tucano, Jatobá⁹ and Sergipe-Alagoas¹⁰ basins. Rift subsidence affected all the basins during the period from Upper Jurassic to Neocomian. A total of 5 and 7 km of non-marine Upper Jurassic to Barremian rift sediments were deposited in the onshore Jatobá and Tucano basins, respectively, over a period of ~24 Myr and there is no evidence of any further significant subsidence. The offshore Sergipe-Alagoas and Gabon^{11,12} basins display 3–4 km of non-marine sediments deposited during the rifting stage. In the Sergipe-Alagoas Basin, rift sedimentation terminated in the Barremian, when it was at a maximum in the onshore basins. The initiation of thermal subsidence within the Sergipe-Alagoas and Gabon basins was marked by a major Aptian/Albian marine transgression¹³ which was approximately contemporaneous with the onset of seafloor spreading, and coincident with the abrupt termination of sedimentation and subsidence within the onshore basins. Following non-marine rift sedimentation in the Sergipe-Alagoas and Gabon basins, 3–4 km of post-rift marine sediments were deposited in response to the thermal subsidence of the margin. Thus, there is an intimate relationship between the differing tectonic histories of the onshore basins (Recôncavo, Jatobá, Tucano) and offshore basins (Sergipe-Alagoas, Gabon, Jacuípe), and the inception of the Atlantic seafloor spreading. The lack of thermal subsidence in the onshore basins confirms the inference from the gravity anomalies that their extension was confined to the upper crust during rifting.

According to the mechanism of McKenzie¹⁴ for the formation of sedimentary basins by uniform lithospheric extension, and more recent modifications^{15,16}, rapid rift-stage subsidence is followed by much slower thermal subsidence, with a time constant between 50 and 150 Myr. The simple McKenzie model fails to explain this coupled system of offshore and onshore basins.

We suggest that all the basins were formed by lithospheric extension during the rifting phase of Atlantic break-up. Upper crustal extension affected both the onshore and offshore basins, but the extension at deeper lithospheric levels, including the lower crust, was concentrated beneath the offshore basins, as evidenced by the degree of thermal subsidence. In order to permit such coupled differential stretching of the lithosphere, the offshore and onshore regions must have been connected by a low-angle crustal detachment surface.

The potential importance of the crustal detachment in controlling basin formation is strengthened by the evolutionary link between onshore and offshore basins. Whereas all of the basins were initiated at the same time, the cessation of rifting terminated onshore basin development at about the same time as the offshore basins entered into the thermal subsidence phase. The detachment has allowed the transfer of extension between the upper crust and the deeper part of the lithosphere during the rifting stage (Fig. 4b). The origin of the onshore Brazilian basins and the Sergipe-Alagoas and Gabon basins conforms to the 'simple shear model' of Wernicke^{4,5}, in which the non-uniform extension of the crust is balanced by lower crustal and sub-crustal lithospheric extension across a detachment surface. Figure 4b shows a schematic but balanced cross-section sum-

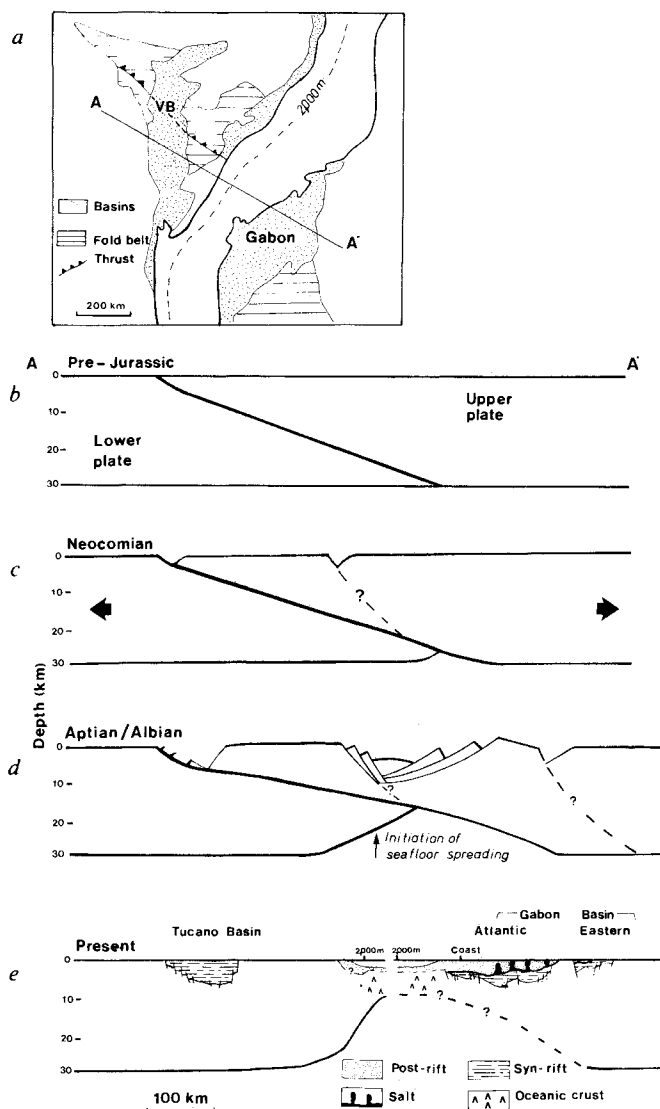


Fig. 4 Suggested history of evolution of the Tucano-Gabon basin system: *a*, Map showing the line of section (A-A') on a pre-drift reconstruction of Brazil and west Africa; VB, Vaza Barris fault. *b*, The postulated contact between the African plate (upper plate) and the São Francisco craton (lower plate) is shown as a pre-existing thrust fault cutting through the crust and possibly the lower lithosphere. *c*, Upper Jurassic-Lower Cretaceous extensional regime allows the upper crust to extend and the basal contact to detach, with complementary extension of the lower part of the lithosphere beneath the incipient marginal basins. The collapse of the hanging wall formed the Tucano basin. *d*, Initiation of seafloor spreading, showing the pronounced thinning of the lithosphere beneath the offshore basins and the asymmetrical position of the split. *e*, Drifting stage, with thermal subsidence of the offshore basins only. Crustal section based on gravity (Tucano and Jacuípe basins), well¹¹ and seismic data¹² (Gabon Basin). The depth of the Moho under the Gabon Basin is only suggested.

marizing the development history of the Brazilian and African basins using the Wernicke model. The upper plate represents the detached thrust block of the Sergipano fold belt and the African craton; the lower plate represents the Brazilian São Francisco craton.

The Tucano and Recôncavo basins cut across the Upper Proterozoic Sergipano fold belt (Fig. 4a), which extends into Gabon and Angola in western Africa². It is possible that the basal thrust plane of this fold belt has been reactivated as an intracrustal detachment during the Mesozoic extension to form the Jatobá and Tucano basins, while synchronous extension and

rifting was taking place further east, with maximum subsidence affecting the Gabon Basin. The final rupture leading to seafloor spreading took place closer to the present Brazilian margin, where only a small thickness of rift sediments had accumulated in the Jacuípe Basin. The exact break-up position is a complicated function of the changing rheological properties of the extending crust and mantle, the evolving lithospheric temperature structure, and any pre-existing crustal fabric^{17,18}.

In plan view, the upper plate has rotated anticlockwise relative to South America^{19,20}, resulting in the observed distribution of basins around its boundaries. In addition to extension leading to basin formation, complementary compression should be induced along the northeastern border of the block. Such compression may be expressed by the major Harmonia and Itapecuru thrust faults along the northeastern segments of the Arcoverde fault²¹ (Fig. 1).

The formation of the entire Jatobá-Tucano-Gabon basin system in the Lower Cretaceous was controlled by a detachment surface. This detachment allowed the onshore Jatobá and Tucano basins to form by brittle failure of the upper crust in extension, without substantial alteration of the thermal structure of the lithosphere beneath the basins, and it has also acted as a mechanical and temporal link between the onshore basins and the continental margins off Brazil and Africa, thereby allowing balancing of lithospheric extension.

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data of the Jatobá and Tucano basins. This work was supported by grants from FAPESP (82/0573-0), CNPq (200348-82), Overseas Research Student Award to N.U. and the Durham University Research Foundation of the Society of Fellows to G.D.K.

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- Schobbenhaus, C., Campos, D. A., Derze, G. R. & Asmus, H. E. *Geological Map of Brazil*, scale 1:2,500,000 (Departamento Nacional da Produção Mineral, Brasília, 1984).
- Allard, G. O. & Hurst, V. J. *Science* **163**, 528-532 (1969).
- Rabinowitz, P. D. & LaBrecque, J. J. *geophys. Res.* **84**, 5973-6002 (1979).
- Wernicke, B. *Nature* **291**, 645-647 (1981).
- Wernicke, B. *Can. J. Earth. Sci.* **22**, 108-125 (1985).
- Nairn, A. E. M. & Stehli, F. G. (eds) in *The Ocean Basins and Margins. The South Atlantic*, 1-20 (Plenum, New York, 1973).
- Rabinowitz, P. D. & Cochran, J. R. *Free-Air Gravity Anomalies of the Continental Margin of Brazil* (American Association of Petroleum Geologists, Tulsa, 1978).
- Kumar, N., Leyden, R., Carvalho, J. & Francisco, O. *Sediment Isopach Map of the Brazilian Continental Margin* (AAPG, Tulsa, 1979).
- Viana, C. F., Gama, E. G. Jr, Simões, I. A., Moura, J. A., Fonseca, J. R. & Alves, R. J. *Bolm. téc. Petrobras* **14**, 157-192 (1971).
- Asmus, H. E. & Ponte, F. C. in *The Ocean Basins and Margins. The South Atlantic* (eds Nairn, A. E. M. & Stehli, F. G.) 87-133 (Plenum, New York, 1973).
- Brink, A. H. *Bull. Am. Ass. petrol. Geol.* **58**, 216-235 (1974).
- Driver, E. S. & Pardo, G. *The Geology of Continental Margins* (eds Burk, C. A. & Drake, C. L.) 293-295 (Springer, New York, 1974).
- Reyment, R. A. & Tait, E. A. *Phil. Trans. R. Soc. B* **264**, 55-95 (1972).
- McKenzie, D. *Earth planet. Sci. Lett.* **40**, 25-32 (1978).
- Royden, L. & Keen, C. *Earth planet. Sci. Lett.* **51**, 343-361 (1980).
- Watts, A., Karner, G. D. & Steckler, M. S. *Phil. Trans. R. Soc. A* **305**, 249-281 (1982).
- England, P. J. *geophys. Res.* **88**, 1145-1152 (1983).
- Sawyer, D. S. *J. geophys. Res.* **90**, 3021-3025 (1985).
- Cohen, C. R. *Bull. Am. Ass. petrol. Geol.* **69**, 65-76 (1985).
- Szatmari, P., Milani, E., Lana, M., Conceição, J. & Lobo, A. *Oil Gas J.* **76**, 107-113 (1985).
- Santos, E. J. *Minerac. Metall.* **313**, 35-40 (1971).

Oldest primitive agriculture and vegetational environments in Japan

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In Japan, shifting agriculture as part of a hunting and gathering strategy was generally thought to have started during the Middle Jomon period (5,000-4,000 BP)¹⁻⁴. We present here evidence of primitive agricultural activities at least 1,600 years earlier than this date. At the Ubuka bog on the coast of the Sea of Japan in southwestern Honshu (34°29' N, 131°35' E, 390 m altitude) buckwheat cultivation dates back to as early as 6,600 BP in the Early Jomon period (7,000-5,000 BP). The warm-temperate climatic regime in that period supported the growth of *Podocarpus*, *Cyclobalanopsis*, *Ilex crenata*/I. *serrata* and *Viscum album* var. *coloratum*. Slash-and-burn agriculture was intensified ~2,000 BP and rice cultivation was introduced ~1,500 BP.

The evidence that slash-and-burn agriculture in the Japanese Archipelago began as part of a generalized subsistence system during the Middle Jomon period is: (1) the abundant occurrence of stone mortars and pestles and chipped stone axes; (2) the establishment of large, permanent villages; and (3) the occurrence of functionally diverse pottery associated with a plant diet¹⁻⁴. However, there has been no direct evidence of agricultural activities, with the exception of nine *Phaseolus aureus* legumes and nine *Lagenaria leucantha* seeds at the Torihama shell mound site³ of the Early Jomon period (Fig. 1).

Fagopyrum esculentum (buckwheat), *Setaria italica* (foxtail millet), *Echinochloa crus-galli* var. *frumentaceum* (Chinese barnyard grass), *Panicum miliaceum* (Chinese millet) and *Oryza sativa* (rice) are all native to the Asiatic mainland⁵⁻¹⁰. These crop plants cannot survive in the moist forested landscape of Japan, unless they are protected by humans, and no pollen grains or seeds of these species have been found in Japanese Pleistocene sediments which have been extensively studied. The

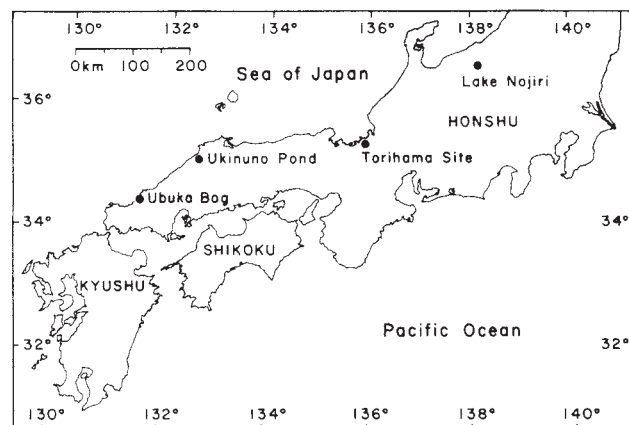


Fig. 1 Index map of sites mentioned in the text.

presence of macroscopic and pollen remains of these plants in Japanese sediments therefore indicates that people were growing them at the time of their deposition¹⁰. Buckwheat, whose pollen can be identified to the species level^{11,12}, is an excellent index species to assess past agricultural activities.

Fossil buckwheat pollen grains were first found in sediments of Lake Nojiri (36°50' N, 138°14' E, 654 m altitude), dated between 1,500 BP and the present¹¹. In these sediments, the pollen of herbaceous taxa, for example *Chenopodiaceae* and *Liguliflorae*, and *Pinus densiflora*, a lowland "pioneer" pine species, begin to increase in frequency immediately after the first occurrence of buckwheat pollen, indicating that shifting agriculture intensified ~1,500 years ago^{10,12}. Some species of these herbaceous taxa can become weeds in agricultural and deserted lands.

Since this first discovery, fossil buckwheat pollen grains have been found at various archaeological sites of the Late Jomon period (4,000-3,000 BP)¹³⁻¹⁵ and a single, carbonized fragment of buckwheat seed has been found on a house floor of the Early Jomon period in southern Hokkaido¹⁶. However, archaeological

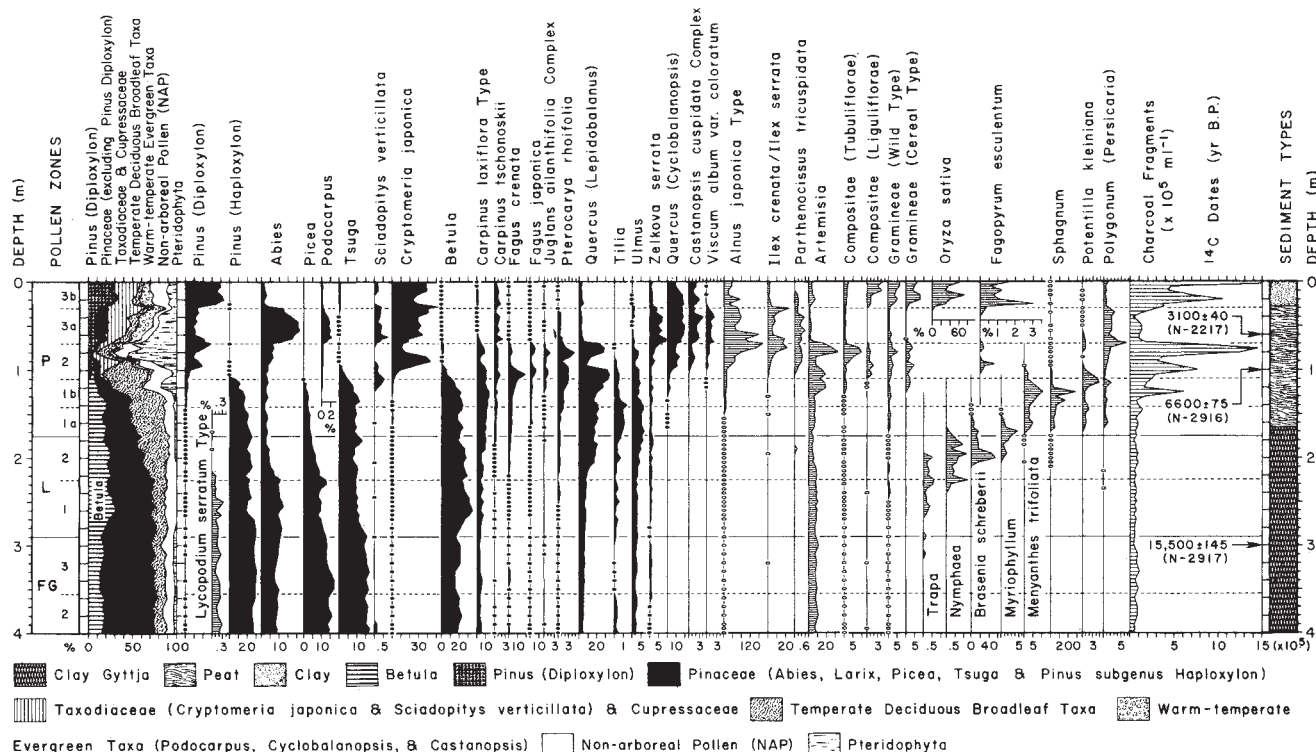


Fig. 2 A pollen diagram of the Ubuka bog, southwestern Japan. Analysis was made at 5-cm intervals from the surface to the 270-cm level, and at 10-cm intervals below the 270-cm level. Percentages are based on the pollen sum (>1,000 grains per level) of upland arboreal taxa, and those listed in the summary diagram (left column) are based on the sum of pollen and spores of terrestrial plants. Dots indicate values of less than 0.2% for *Lycopodium* and *Tilia*, 0.3% for *Carpinus latifolia* type, *Juglans*, *Pterocarya*, *Viscum*, *Compositae* (Liguliflorae), *Myriophyllum*, *Potentilla*, and *Persicaria*, 0.5% for *Abies*, *Picea*, *Tsuga*, *Zelkova*, *Menyanthes*, and *Compositae* (Tubuliflorae), 1.0% for *Pinus*, *Cryptomeria*, *Betula*, *Fagus*, *Quercus*, *Carpinus laxiflora* type, *Gramineae* and *Ilex*, and 5% for *Alnus*.

sites are often disturbed by past occupations, making it impossible to reconstruct the history of fossil pollen and seeds precisely, and one seed is not a conclusive demonstration that buckwheat cultivation was practised at a particular site¹⁶. In contrast to this, bog and lake sediments are well stratified and can provide a reliable picture of buckwheat history.

We have now found definitive evidence of primitive agriculture at the Ubuka bog shown in Fig. 1 (1,000 m N-S × 1250 m E-W). During the full- and late-glacial period the basin was a lake, characterized by gytja sediments with cladoceran remains, but it was gradually obliterated by sediment accumulation between ~13,000 and 8,500 BP. All dates are based on three ¹⁴C datings [3,100 ± 90 BP at 60 cm (Nippon Isotope Association sample number N-2217), 6,600 ± 75 BP at 100 cm (N-2916), and 15,500 ± 175 BP at 300 cm (N-2917)] and the correlation with dated pollen zonal boundaries in southwestern Japan. A well-ordered succession from shallow-lake aquatic plants (*Trapa*, *Nymphaea tetragona*, *Brasenia schreberii*, *Myriophyllum* and *Menyanthes trifoliata*) to bog plants (*Sphagnum*, *Potentilla kleiniana* and *Persicaria*) is evidence of this change (Fig. 2).

About 7,700 years ago, or soon after the bog was formed, the forest surrounding the basin was disturbed. This is indicated by a sudden increase in the deposition of charcoal fragments of woody plants at this time. These fragments are large in size but make up only 1.1% of the total (Fig. 3). Grass pollen grains found in these strata have not been identified at the species level but many are >41 μm (Fig. 2), suggesting that cereal plants¹⁷⁻²⁰ may have been under cultivation in the Early Jomon period. No grass pollen >35 μm occurred in sediments accumulated before ~7,500 BP.

The deposition of charcoal fragments increased further ~6,600 BP. Some of these fragments are 150–400 μm (Fig. 3), indicating that forest fires occurred nearby. Buckwheat pollen

grains were found in abundance (75.8 ± 61.7 cm⁻³) in sediments dated ~6,600–4,500 BP. Herbaceous 'pioneers', for example *Artemisia* and other *Compositae*, are also conspicuous during this period and pine (mostly *P. densiflora*) reaches a frequency of 28% total pollen. It is unlikely that buckwheat was cultivated on the Ubuka bog at this time because no peat disturbance was observed and *Alnus japonica* and *Ilex crenata*/*I. serrata* actively invaded the bog during this period (Fig. 2).

Even after a considerable search of the Ubuka sediments, neither buckwheat nor cereal pollen were found in the sediments deposited between ~4,500–2,000 BP, approximately corresponding to pollen zone P-3a time²¹ (Fig. 2), probably because agricultural activities were moved to sites at a distance from the bog. Temperate conifers (*Abies firma* and *Cryptomeria japonica*) increased in frequency immediately after agricultural activities ceased. Carbon fragments became smaller and the Kolmogorov-Smirnov two-sample test²² shows that, at the 0.01 significance level, their frequency curve is identical to those of distant fires recorded at the 230-cm level (~12,400 BP) and other late- and full-glacial levels not shown in Fig. 3. Heavy charcoal and buckwheat pollen should have not been carried from the distant sites to the bog during the P-3a zone. However, a relatively high frequency of *Diploxylon* pine pollen during this period indicates that human groups still continued to disturb the forests distant from the bog (Fig. 2).

The primitive agricultural activities approximately correspond to the Japanese Hypsithermal interval, or pollen zone P-2 time (7,000–4,000 BP)^{21,23}. By ~7,000 BP, populations of many cool-temperate species, for example spruce (*Picea*), basswood (*Tilia*) and birch (*Betula*), declined in frequency or disappeared completely from the Ubuka area. A rapid decline of beech (*Fagus*) and oak (*Quercus*) pollen ~6,600 BP suggests that forest fires may have killed many trees around the Ubuka bog and that

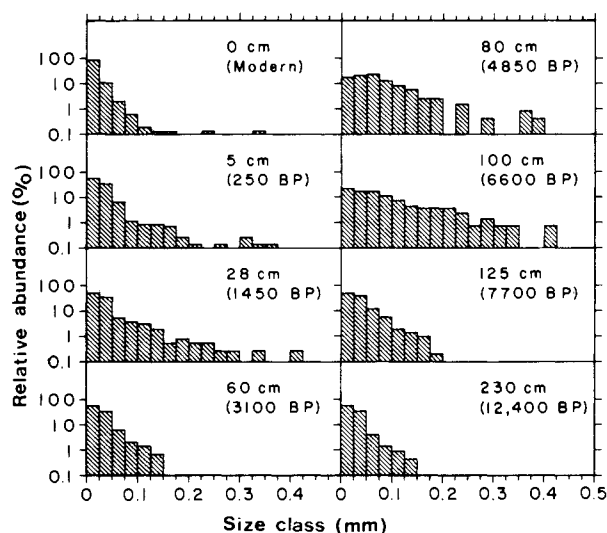


Fig. 3 Frequency curves of charcoal fragments at selected levels of the Ubuka core. *N* (total number fragments counted) = 224 at level 230 cm; 621 at 125 cm; 261 at 100 cm; 245 at 80 cm; 294 at 60 cm; 357 at 28 cm; 797 at 5 cm; 1,593 at 0 cm. Approximate dates, calculated from three radiocarbon dates (Fig. 2) and dated pollen zonal boundaries^{21,28}, are given in brackets.

after these disturbances Pteridophyta (which includes bracken) characterized the Early Jomon landscape. Also common were warm-temperate species such as *Podocarpus* (which can grow in areas above the 2 °C January isotherm²⁴) and *Cyclobalanopsis* (cyclic cup oak, a subgenus of *Quercus*).

Cryptomeria japonica, a temperate moisture-loving species which requires at least 1,600 mm of annual precipitation^{25,26}, began to increase at the beginning of pollen zone P-2 time. Both this species and *Sciadopitys verticillata* declined temporarily ~5,000–4,000 BP as a result of forest disturbances. *Ilex* pollen is unusually high (6–20% of total tree pollen) during this period. Pollen of this entomophilous genus would not be found in such abundance unless the swamp species, either *Ilex crenata*, *I. serrata*, or both, had invaded the bog 7,000 BP. A more important finding is that Japanese mistletoe (*Viscum album* var. *coloratum*) arrived at this area ~7,500 BP and has continued to grow up to the present day. Although the climatic factors under which this species can grow and reproduce sexually have not been studied (as has been done for the European species²⁷), its presence together with *Podocarpus* suggests a warm winter climate.

About 2,000 years ago, buckwheat was again cultivated near the bog; people have continued to grow it here until the present day. The buckwheat pollen frequency for the past 2,000 years is much higher than that observed during the first period of cultivation. Large charcoal fragments are also observed at all analytical levels deposited during this period. The fragments >150 µm are 16.9% at level 100 cm (~6,600 BP), 9.4% at 80 cm (4,850 BP), 3.6% at 28 cm (1,450 BP), 1.8% at 5 cm (250 BP) and 0.4% at 0 cm (Fig. 3). The frequency for the upper levels is low because small charcoal fragments were carried from distant agricultural areas to the bog and further fragmented by farming activities on the paddy field. These facts suggest that much more intensified slash-and-burn agriculture has been practised in the area for the past 2,000 years.

Although the clay sediments above the 28-cm level were disturbed by rice cultivation on the bog, pollen succession of upland plants here is almost identical to that established at the nearby Ukinuno Pond site²⁸ about 127 km northeast of Ubuka (Fig. 1). At Ubuka, however, buckwheat pollen was found in an undisturbed peat layer, indicating that intensified shifting agriculture began slightly before the disturbance of the bog caused by wetland rice cultivation. By interpolating the sedimentation rate between the modern level and the level radiocarbon-dated at 3,100 ± 90 yrs, the date of the first rice pollen can be estimated at about 1,500 BP. This is within the period when rice agricultural practice was intensified along the coast of the Sea of Japan in southwestern and central Honshu²⁸.

These intensified agricultural activities almost completely

destroyed the temperate *Abies* and *Cryptomeria* forests at the Ubuka area. Red pine increased further and currently comprises ~40% of total tree pollen. Among the herbaceous pollen, an increase of *Liguliflorae* is also conspicuous; its abundance level is higher than that observed during the earlier agricultural episode. Since rice cultivation was practised on the bog, plants such as *Ilex* and *Alnus* have sharply declined in abundance (Fig. 2).

The presence of fossil buckwheat pollen grains and the sudden increase of charcoal fragments at the Ubuka basin ~6,600 radiocarbon years ago provide evidence of agricultural activities in Japan at least 1,600 years earlier than the previously inferred date. Buckwheat was introduced from central China, which is considered to be the probable centre of buckwheat cultivation^{5–9}. The coast of the Sea of Japan in southwestern Japan, directly facing mainland Asia, is a likely place for shifting agriculture to have begun. Further extensive studies along this line, with detailed pollen and charcoal analyses, are needed to clarify the existence of earlier agriculture and its migration routes both in East Asia and within the Japanese Archipelago.

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1. Ezaka, T. *The Origin of Japanese Culture* (Kodansha, Tokyo, 1967).*
2. Fujimori, E. *The Jomon Culture* (Gakuseisha, Tokyo, 1970).*
3. Sasaki, K. *Before Rice Agriculture* (Nippon Hoso Kyokai, Tokyo, 1971).*
4. Sasaki, K. in *Rice and Iron: Foundation of Various Regal Powers* (eds. Amino, Y., Ohbayashi, T., Takatori, M., Tanikawa, K., Tsuboi, H., Miyata, N. & Mori, K.) 56–130 (Shogakukan, Tokyo, 1983).*
5. Vavilov, N. I. *The Origin, Variation, Immunity and Breeding of Cultivated Plants* (Ronald, New York, 1951).
6. Bailey, L. H. *Hortus Third* (MacMillan, New York, 1976).
7. Shiotani, I. *The History of Cultivated Plants* (Hosei Univ. Press, Tokyo, 1977).*
8. Hoshikawa, K. *The Origin and Spread of Cultivated Plants* (Ninomiya-shoten, Tokyo, 1978).*
9. Nakao, S. *Cultivated Plants and the Origin of Agriculture* (Iwanami-shoten, Tokyo, 1966).*
10. Tsukada, M. *Bot. Mag., Tokyo* **80**, 323–336 (1967).*
11. Ananova, Y. N. *Dokl. (Proc.) Acad. Sci. U.S.S.R.* **128**, 165–167 (1959).
12. Tsukada, M. *Bot. Mag., Tokyo* **79**, 179–184 (1966).*
13. Miyoshi, N. & Usui, Y. in *Palynological Studies on the Origin and Migration of Rice Agriculture* (ed. Nakamura, J.) 30–35 (Kochi University, Kochi, 1977).*
14. Yamanaka, M. *Ecol. Rev., Tohoku Univ.* **19**, 113–121 (1979).
15. Nakamura, J. *Proc. 4th Int. Palynol. Conf. Lucknow*, **3**, 238–247 (1981).
16. Crawford, G. W., Hurley, W. M. & Yoshizaki, M. *Asian Perspectives* **19**, 145–155 (1976).
17. Firbas, F. Z. *Bot. Z.* **31**, 447–478 (1937).
18. Beug, H.-J. *Leitfaden der Pollenbestimmung* (Fischer, Stuttgart, 1961).
19. Tsukada, M. *Pollen Spores* **6**, 393–462 (1964).
20. Nakamura, J. *Daiyoni-kenkyu (Quat. Res.)* **13**, 187–193 (1974).*
21. Tsukada, M. in *Vegetation of Japan VII. Kanto* (ed. Miyawaki, A.) 78–103 (Shibundo, Tokyo, 1986).*
22. Campbell, R. C. *Statistics for Biologists* (Cambridge University Press, 1974).
23. Tsukada, M. *Jap. J. Ecol.* **32**, 113–118 (1982).
24. Tsukada, M. in *Encyclopedia of Japan* **7**, 177–179 (Kodansha, New York, 1983).
25. Tsukada, M. *Am. J. Bot.* **54**, 821–831 (1967).
26. Tsukada, M. *Quat. Res.* **26**, 135–152 (1986).
27. Iversen, J. *Geol. Foren. Forhandl.* **66**, 463–483 (1944).
28. Tsukada, M. in *Windows on Japanese Past: Studies in Archaeology and Prehistory* (ed. Pearson, R. J.) 11–56 (University of Michigan, Press, Ann Arbor, 1986).

* In Japanese.

Olfactory dysfunction in humans with deficient guanine nucleotide-binding protein

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The guanine nucleotide-binding stimulatory protein (G_s) couples hormone–receptor interaction to the activation of adenylate cyclase and the generation of cyclic AMP (see ref. 1 for a review). Studies using frog neuroepithelium indicate that the sense of smell is mediated by a G_s –adenylate cyclase system², and this prompted us to test olfaction in the only known model of G_s deficiency in the animal kingdom, G_s -deficient (type 1a) pseudohypoparathyroidism (PHP), which occurs in humans^{3–8}. Such patients are resistant to the cAMP-mediated actions of several hormones⁹. (Although Henkin¹⁰ has reported disturbances in the sense of smell in six patients with PHP, currently available biochemical measurements such as the cAMP response to parathyroid hormone (PTH) and determination of G_s activity were not reported and olfactory

testing was limited.) In the present study, we found that all G_s -deficient patients had impaired olfaction when compared with PHP patients who had normal G_s activity (type 1b PHP, in which patients are resistant only to the action of PTH in the kidney). This is the first evidence of human olfactory impairment which can be related to G_s deficiency and suggests that G_s -deficient PHP patients may be resistant to cAMP-mediated actions in other non-endocrine systems.

We tested olfaction in five patients with type 1a PHP, who typically express variable resistance to the cAMP-mediated actions of several hormones^{3,9,11–13} and in seven patients with type 1b PHP, who are not resistant to the action of other hormones whose activities are mediated via cAMP⁹. Informed consent was obtained. The diagnosis of PHP was based on the information shown in Table 1. All PHP patients had hypocalcaemia with elevated PTH levels at the time of diagnosis, normal renal function, and all required therapy with calcium and vitamin D. Nine of the 12 patients were infused with 250 U of parathyroid extract and had markedly impaired urinary cAMP responses. All five type 1a PHP patients had deficient erythrocyte G_s activity and features of Albright's osteodystrophy. All were resistant to thyroid stimulating hormone (TSH) with elevated basal or thyrotropin releasing hormone-stimulated TSH levels, and all require treatment with thyroid hormone (T_4) for hypothyroidism. These five patients all had negative titres of anti-thyroid antibodies, suggesting that such patients are demonstrating thyroid resistance to TSH for reasons other than autoimmune thyroid disease. At the time of olfaction testing, all PHP patients were being treated with calcium and vitamin D and all the type 1a patients were also receiving supplements of thyroid hormone. This is important because several endocrine deficiencies, including hypothyroidism, have been reported to be associated with olfactory dysfunction¹⁴. Serum calcium, phosphorus,

Table 1 Pretreatment characteristics of patients with pseudohypoparathyroidism

Patient no.	Age (yr)	Sex	Untreated serum calcium (mg dl ⁻¹ ; low limit of normal = 8.4)	Untreated serum phosphorus (mg dl ⁻¹ ; upper limit of normal = 4.8)	Serum PTH when hypocalcaemic†	Peak urinary cAMP response to PTH (nmol per mg Cr; normal 90–315)	G_s activity (% of normal; normal > 75%)
Type 1a PHP patients							
1	37	F	7.2*	4.8*	Elevated	ND	55
2	26	F	8.0	4.9	Elevated	8.80	66
3	30	F	8.2	4.6	Elevated	ND	48
4	36	F	6.6	4.3	Elevated	4.25	51
5	20	F	6.0	7.1	Elevated	7.0	51
Type 1b PHP patients							
6	60	F	5.5	3.3	Elevated	4.16	87
7	47	F	6.3	ND	Elevated	15.83	85
8	30	F	6.4	4.7	Elevated	5.07	78
9	31	F	6.8	5.6	Elevated	4.08	ND; normal phenotype, sister of patient 8
10	38	F	7.2*	4.3*	Elevated	‡	ND; normal phenotype
11	38	F	6.8	4.4	Elevated	ND	78
12	22	M	8.4*	5.1*	Elevated	6.0	88

Erythrocyte G_s activity was determined as previously reported^{5,8}. Blood was anticoagulated with acid citrate dextrose and erythrocyte membranes were prepared within 48 h of collection. Erythrocyte membranes were prepared by hypotonic lysis in 5 mM sodium phosphate (pH 8.0) at 0 °C and stored at –70 °C. G_s activity was extracted from erythrocyte membranes in detergent and assayed by addition to CYC^- membranes. Membranes from the CYC^- clone (94-15-1) of S49 mouse lymphoma cells, genetically deficient in G_s activity¹⁶, were prepared as described previously¹⁷. Erythrocyte membrane pellets were prepared by centrifugation and solubilized in buffer A (2.0 mg protein ml⁻¹, 10 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 0.1 mM EDTA, 1 mM dithiothreitol, 0.2% (w/v) Lubrol PX) and incubated at 4 °C for 60 min with frequent vortexing. The membrane suspensions were then centrifuged for 3 min at 50,000g and the supernatant solutions (membrane extract) collected and retained at 0–4 °C for immediate assay of G_s activity. CYC^- membranes (200 µg) and soluble erythrocyte membranes (10 µg) were incubated for 20 min at 30 °C in 80 µl of buffer B containing 30 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, 20 µM cAMP, 5 mM creatine phosphate, 3.3 IU of creatine phosphokinase, 100 µM ATP, isoprenaline (10 µM) and guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S; 100 µM). After the 20-min incubation, 20 µl of buffer B containing [α -³²P]ATP (0.1 Ci mmol⁻¹) was added and incubation continued for a further 20 min at 30 °C. cAMP was isolated and determined by the method of Salomon *et al.*¹⁸. Erythrocyte extracts alone did not show measurable adenylate cyclase activity. Adenylate cyclase activity of CYC^- membranes in response to isoprenaline/GTP-γ-S (1.0–1.5 pmol cAMP per tube) was subtracted from the activity of combined erythrocyte extract and CYC^- membranes to obtain the increment due to addition of erythrocyte G_s activity. Results of assays are expressed in relation to the activity of a standard membrane preparation consisting of pooled erythrocytes from four normal persons and represent the mean of three determinations. ND, not done.

* Values obtained when the patient was receiving treatment with calcium and vitamin D.

† Serum PTH determinations were performed by four different research or reference laboratories.

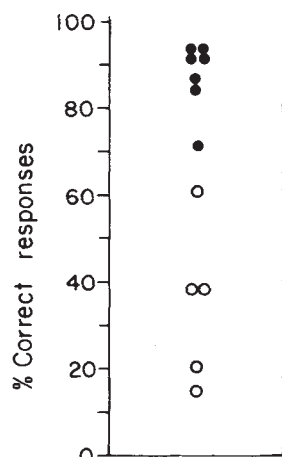
‡ Plasma cAMP = 25 pmol ml⁻¹ (normal > 150).

Table 2 Serum values at the time of olfaction testing

Patient no.	Calcium (mg dl ⁻¹ ; low limit of normal = 8.4)	Phosphorus (mg dl ⁻¹ ; upper limit of normal = 4.8)	T ₄ (μg dl ⁻¹ ; lower limit of normal = 5)	TSH (μl ml ⁻³ ; upper limit of normal = 6)
Type 1a PHP patients				
1	9.0	3.6	4.5	1.6
2	9.0	4.0	7.4	7.8
3	8.8	3.4	7.7	3.8
4	9.8	4.3	8.6	3.3
5	9.6	4.0	9.3	<0.1
Type 1b PHP patients				
6	8.4	4.3	9.2	3.4
7	9.2	3.7	5.1	5.2
8	8.0	4.0	5.8	3.5
9	9.2	3.6	6.4	5.3
10	7.2	4.3	7.1	13.1*
11	9.4	3.9	5.2	1.8
12	8.4	5.1	6.4	2.6

All G_s-deficient patients were receiving therapy with calcium, vitamin D and thyroid hormone. All PHP patients with normal G_s activity were receiving therapy with calcium and vitamin D.

* Anti-thyroid antibodies present.

**Fig. 1** Results of olfaction testing in patients with type 1a PHP (open circles) and patients with type 1b PHP (closed circles).

Methods. Each patient was evaluated by asking them to identify repeatedly 10 common odorants presented at concentrations found to be representative of their counterparts in nature, which frequently are odorant mixtures rather than single pure chemicals: 3.6015 M ammonia (ammonia), 0.0622 M *trans*-cinnamaldehyde (cinnamon), 0.0130 M anethole (licorice), 0.3990 L-carvone (mint), 0.0244 M naphthalene (mothballs), 0.0121 M D-limonene (orange), 1.0467 M phenethyl alcohol (rose), 0.7425 M isopropyl alcohol (rubbing alcohol), 0.0273 M vanillin (vanilla), and 4.3714 M acetic acid (vinegar)¹⁵. The diluent was odourless propylene glycol. The odorants were successively presented with the sniff-bottle technique randomly for each subject in blocks of 10, with the constraint that when moving continuously from one block to the next, no one odorant followed itself. The subjects were informed of the odorants which were to be presented and could use any sniffing strategy they desired, but were encouraged to make their identification decision after about two sniffs. It has been shown that a single natural sniff is usually sufficient for optimum odorant perception, and that additional sniffs are probably only confirmatory¹⁹. The results are expressed as the percentage of 100 odorant presentations (10 for each odorant) identified correctly¹⁵.

T₄, TSH and PTH values at the time of testing are shown in Table 2. No patients had upper respiratory infections or other active nasal problems that could influence their sense of smell.

Olfaction was evaluated by a quantitative test which required patients to identify 10 common odours that were repeatedly presented in a random fashion¹⁵. Figure 1 shows the results. All five type 1a PHP patients demonstrated impaired olfaction compared with the seven type 1b PHP patients. One of the latter group had quantitative olfactory dysfunction when compared with normal subjects, but even this patient scored much better on olfactory testing than any of the type 1a patients.

Our study shows that G_s deficiency is associated with decreased olfactory ability and provides the first evidence of a mechanism involving G_s-adenylate cyclase in human olfaction. It further demonstrates that PHP, a disease traditionally thought to be characterized by endocrine dysfunction, may involve other organ systems in which specific effects are mediated via cAMP.

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1. Spiegel, A. M., Gierschick, P., Levine, M. A. & Downs, R. W. Jr *New Engl. J. Med.* **312**, 26-33 (1985).
2. Pace, U., Hanski, E., Salomon, Y. & Lancet, D. *Nature* **316**, 255-258 (1985).
3. Spiegel, A. M. *et al. Am. J. Physiol.* **243**, E37-E42 (1982).
4. Farfel, Z., Brickman, A. S., Kaslow, H. R., Brothier, V. M. & Bourne, H. R. *New Engl. J. Med.* **303**, 237-242 (1980).
5. Levine, M. A. *et al. Biochem. biophys. Res. Commun.* **94**, 1319-1324 (1980).
6. Farfel, Z., *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 3098-3102 (1981).
7. Spiegel, A. M. & Downs, R. W. Jr *Endocr. Rev.* **2**, 275-305 (1981).
8. Levine, M. A., Eil, C., Downs, R. W. Jr & Spiegel, A. M. *J. clin. Invest.* **72**, 316-324 (1983).
9. Levine, M. A. *et al. Am. J. Med.* **74**, 545-556 (1983).

10. Henkin, R. I. *J. clin. Endocr. Metab.* **28**, 624-628 (1968).
11. Marx, S. J., Hershman, J. M. & Aurbach, G. G. *J. clin. Endocr. Metab.* **33**, 822-828 (1971).
12. Werder, E. A. *et al. Pediatr. Res.* **9**, 12-16 (1975).
13. Wolfson, J. I. *et al. Acta endocr. Copenh.* **88**, 321-328 (1978).
14. McConnell, R. J., Menendez, C. E., Smith, F. R., Henkin, R. I. & Rivlin, R. S. *Am. J. Med.* **59**, 354-364 (1975).
15. Wright, H. N. *Archs Otolar.* (in the press).
16. Ross, E. M., Howlett, A. C., Ferguson, K. M. & Gilman, A. G. *J. biol. Chem.* **253**, 6401-6412 (1978).
17. Nielsen, T. B., Ladd, P. M., Preston, S. J. & Rodbell, M. *Biochim. biophys. Acta* **629**, 143-155 (1980).
18. Salomon, Y., Lonzo, C. & Rodbell, M. *Analyt. Biochem.* **58**, 541-548 (1974).
19. Laing, D. G. *Perception* **12**, 99-117 (1983).

Synthesis of fast myosin induced by fast ectopic innervation of rat soleus muscle is restricted to the ectopic endplate region

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Skeletal muscle fibres, long multinucleated cells, arise by fusion of mononucleated myoblasts to form a myotube that matures into the adult fibre. The two major types of mature fibre, fast and slow fibres, differ physiologically in their rate of isotonic shortening¹. At the molecular level these type-specific physiological properties are ascribed to different isoforms of myosin, a major protein involved in shortening^{2,3}. Differentiation of fast and slow fibres seems to be under the control of motoneurons⁴, and mature fibres are innervated by only one motoneurone. When rat soleus muscle (SOL, a slow muscle) is dually innervated with a fast nerve, it acquires some properties of a fast muscle, that is, low sensitivity to caffeine and high glycogen content⁵. We report here that in dually innervated soleus muscle the foreign fast nerve induces synthesis of fast isoforms of myosin, but only in the segment of the muscle fibre that is close to the foreign endplate. The localized influence of the nerve endplates suggest that factors controlling the phenotypic expression of the muscle fibre have a short range of activity.

The nerve of the fast extensor digitorum longus (EDL) muscle of male Wistar rats (each weighing 160 g) was transposed and inserted with the aid of a glass capillary under the fascia of the soleus muscle, in the proximal third of the muscle. Care was taken not to damage the muscle fibres underneath. After 3 weeks, the SOL was temporarily denervated by crushing and freezing its motor nerve with clamps that had been immersed in liquid nitrogen. This procedure enables the soleus muscle fibres to be innervated rapidly (as early as 3 days after nerve crushing⁶) by the fast nerve, which forms new endplates that are functionally normal and remain active for long periods, perhaps indefinitely⁷. Also, after 2 weeks the normal nerve re-establishes contacts with the target fibres at the original neuromuscular junctions⁷. We studied the dually innervated SOL either 3 or 14 months after surgery, electrically stimulating the muscles via the nerve to be certain that both normal and foreign nerves could induce contraction. All operated muscles ($n = 10$) contracted on stimulation of either nerve. We dissected the operated soleus muscle, and, as a control, the contralateral muscle, and mounted them on a wooden stick, then chemically skinned the fibres to extract soluble sarcoplasmic proteins that would interfere with the identification of myosin light chains and regulatory proteins in the gels⁸.

To identify dually innervated fibres, we treated bundles of muscle fibres with a skinning solution (170 mM K-propionate, 2.5 mM Mg propionate, 2.5 mM ATP, 5 mM EGTA, 10 mM imidazole-propionate, pH 7.0) containing 10 μ M α -bungarotoxin labelled with fluorescein isothiocyanate, a reagent that binds to the acetylcholine receptors. In both control and experimental muscle fibres, α -bungarotoxin was localized exclusively at the endplates. We dissected single fibres and examined them by fluorescence microscopy to identify those with two endplates. These were found in the proximal end (1–2 mm from tendon insertion, ectopic endplate) and in the middle region of the muscle fibre (5–6 mm from the ectopic endplate) where endplates are normally localized. We solubilized fibres in SDS-containing solution and analysed myofibrillar protein isoforms by SDS-gel electrophoresis on a discontinuous

Table 1 Localization of fast MHC isoforms in single soleus fibres bearing two separate endplates

		Proximal end Ectopic endplate		Original endplate	Distal end
Segment no.		1	2	3	4
Three-months operated					
Fibre no. 1	F	+	—	—	—
	S	++	+++	+++	+++
2	F	+	—	—	—
	S	++	+++	+++	+++
3	F	+	—	—	—
	S	++	+++	+++	+++
4	F	+	—	—	—
	S	++	+++	+++	+++
5	F	+	—	—	—
	S	++	+++	+++	+++
6	F	+	—	—	—
	S	++	+++	+++	+++
7	F	+	—	—	—
	S	++	+++	+++	+++
Fourteen-months operated					
Fibre no. 1	F	++	+	—	—
	S	+	++	+++	+++
2	F	+	—	—	—
	S	++	+++	+++	+++
3	F	++	+	—	—
	S	+	++	+++	+++
4	F	++	—	—	—
	S	+	+++	+++	+++
5	F	++	—	—	—
	S	+	+++	+++	+++
6	F	++	+	—	—
	S	+	++	+++	+++
7	F	++	—	—	—
	S	+	++	+++	+++
8	F	++	—	—	—
	S	+	+++	+++	+++
9	F	++	+	—	—
	S	+	++	+++	+++
10	F	+	—	—	—
	S	++	+++	+++	+++
11	F	++	+	—	—
	S	+	++	+++	+++
12	F	++	—	—	—
	S	+	+++	+++	+++
13	F	++	—	—	—
	S	+	+++	+++	+++
14	F	++	+	—	—
	S	+	++	+++	+++
15	F	++	+	—	—
	S	+	++	+++	+++
16	F	+	—	—	—
	S	++	+++	+++	+++
17	F	++	—	—	—
	S	+	+++	+++	+++
18	F	++	—	—	—
	S	+	+++	+++	+++

Single muscle fibres, showing two separate endplates after α -bungarotoxin labelling, were isolated from 3-months (4 muscles) or 14-months (6 muscles) operated soleus muscles. Each fibre was cut into four segments (each 2 mm long starting from the proximal end). Fast (F) and slow (S) myosin heavy chains were separated by 5% SDS-gel electrophoresis and stained with silver. (+) indicates the amount of fast and slow MHC in each fibre segment. Fibre segments were 2 mm long.

system⁹ as described previously^{8,10}.

The presence of two separate endplates in the same fibre may not be an absolute criterion for dual (fast and slow) innervation. It has been reported that in some instances branches of the same foreign motor nerve form separated endplates on the same muscle fibre when the original nerve was cut and prevented from reinnervating the muscle¹¹. Furthermore, many denervated

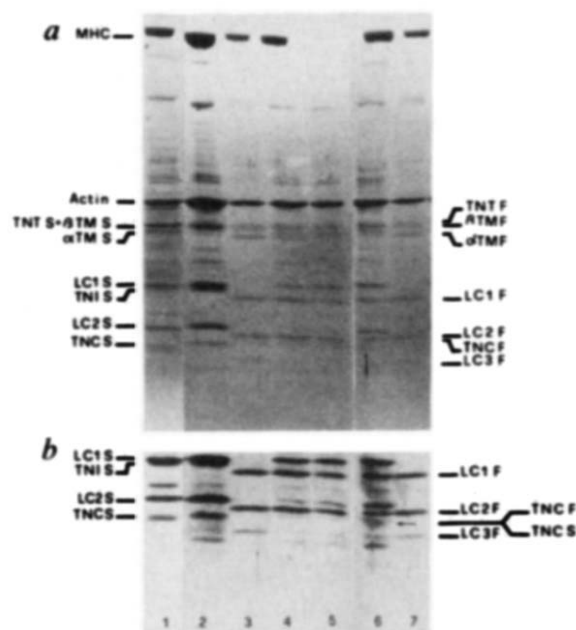


Fig. 1 Pattern of myofibrillar proteins of single fibres from operated soleus muscle. SDS-gel electrophoresis on 10–20% polyacrylamide linear gradient of chemically skinned single-fibre proteins. Lanes 1, 2, control slow fibres from SOL muscle; 3, 7, control fast fibres from EDL muscle; 4–6, dually-innervated soleus fibres identified by fluorescent α -bungarotoxin labelling (see text). In lane 5, MHC material is missing because of a cracking in the gel. After electrophoresis the gel was stained with Coomassie Brilliant Blue (panel *a*), destained and stained with silver (panel *b*); in *b* only the region of MLC is shown. F, fast; S, slow; MHC, myosin heavy chain; LC, myosin light chain; TNT, TNI, TNC, troponin T, I or C; TM, tropomyosin.

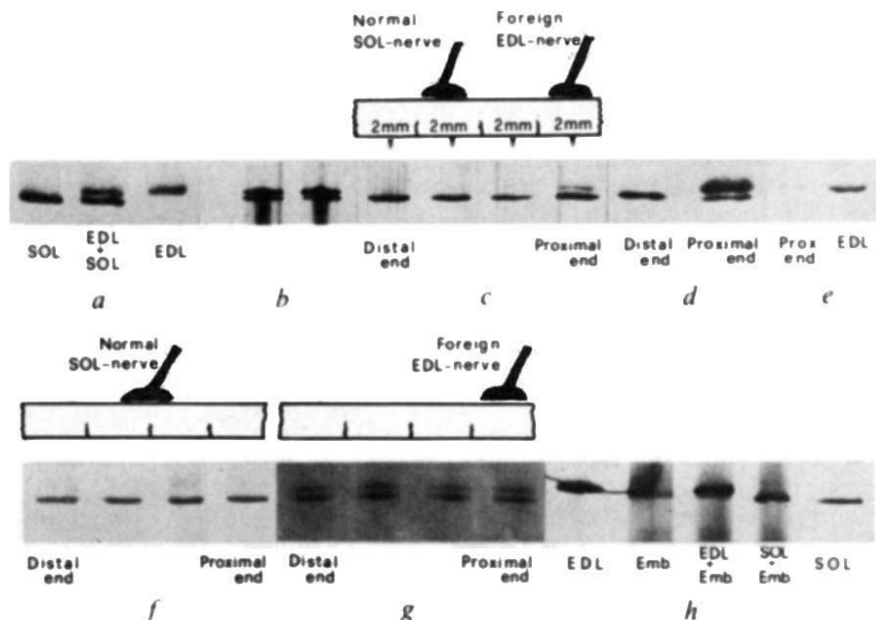
endplates of muscle cross-innervated by a foreign nerve at a distant location continue to bind normal amounts of α -bungarotoxin for more than 4 months¹². However, at later stages (30–40 weeks) after denervation of the original nerve, a decreased acetylcholine sensitivity has been demonstrated in non-reinnervated original endplates⁷. Most of our fibres were analysed 14 months after the operation and no differences were found in the intensity of α -bungarotoxin fluorescence between ectopic and original endplates. This result and the fact that operated soleus muscles contracted to electrical stimulation of either normal or foreign nerve suggest, although do not prove, that most of the original endplates were reinnervated by the normal nerve⁷. The possibility still exists that not all the fibres studied were in fact dually innervated.

Fast and slow myosin light chains (MLC), tropomyosin (TM) and troponin (TN) showed different apparent relative molecular mass (Fig. 1; lanes 3, 7 and 1, 2, respectively). Fourteen months after surgery, all experimental soleus fibres ($n = 13$) contained

both fast and slow isoforms of MLC, TM and TN (Fig. 1; lanes 4–6), indicating that the foreign fast nerve could activate synthesis of fast isoforms of MLC and the regulatory proteins. Fast and slow rat myosin heavy chains (MHC) can be identified by an electrophoretic gel system that requires very little protein (ref. 13; Fig. 2*a*), making it possible to study the composition of MHC in single fibres. In all experimental fibres ($n = 13$), we found both fast and slow MHC (Fig. 2*b*), although the relative proportion of the two isomyosins varied. Furthermore, the additional protein band which appeared in experimental fibres was selectively immunostained by a monoclonal antibody specific for adult fast MHC (Fig. 2*e*). These findings indicate that a fast nerve implanted onto a normally innervated slow fibre modify the 'trophic' effect of the slow nerve, activating genes encoding fast myosin.

Our results, however, raise another question: whether the effect of the fast nerve was exerted on the entire fibre or only near the fast endplate. To answer this question, we cut experi-

Fig. 2 Expression of fast myosin heavy chains by single fibres from operated soleus muscle. MHC (~50 ng protein) were separated by SDS-gel electrophoresis on 5% polyacrylamide gel and stained with silver. *a*, Control muscles; *b*, total protein of two dually innervated fibres (see Fig. 1); *c*, dually innervated fibre 3 months after surgery—4 fibre segments of the same length (2 mm) were cut as shown by the upper scheme and separately electrophoresed; *d*, dually innervated fibre 14 months after surgery—the fibre was cut into two halves about 4 mm long, that were separately electrophoresed; *e*, dually innervated fibre cut as in *d* and EDL control fibre after blotting²³ and immunostaining with anti-rat fast MHC monoclonal antibody (24D5); *f*, contralateral, control, soleus muscle fibre—four segments (2 mm long) were cut and processed as *c*; *g*, fibre (10 mm long) from 14-months operated soleus. This fibre, after fluorescent α -bungarotoxin labelling, showed only the foreign endplate at the proximal end—MHC were analysed in four segments as shown in *c*; *h*, rat embryonic MHC does not co-migrate with adult fast and slow MHC. Embryonic myosin was purified from 12 days rat fetus hind limb muscles according to ref. 24.



mental fibres into four segments (2 mm long) from the proximal end, where the fast endplate was located. Although fast MHC are found in all fibres 3 or 14 months after surgery (Fig. 2C-D; Table 1), they are detected almost exclusively in the segment of fibre that bears the fast endplate and no fast MHC is ever detected in the segment containing the slow endplate or that located more distally. Only the proximal segment of six 14-month dually innervated fibres is stained by histochemical myofibrillar ATPase reaction, after precubincubation at pH 10.4, that is, after inhibition of slow myosin ATPase activity (data not shown). Normal soleus fibres contained only slow MHC, even in the segment corresponding to the proximal end (Fig. 2f). By contrast, a fibre from a single 14-month operated soleus muscle, in which only the foreign endplate at the proximal end was demonstrated by the α -bungarotoxin labelling, contained both fast and slow MHC, but the relative amounts of the two isoforms were constant throughout the entire fibre length (Fig. 2g).

We conclude that this fibre is a slow fibre 'cross-innervated' by the fast nerve that undergoes a partial slow-to-fast transformation, in agreement with previous results¹⁴. The possibility that the additional band seen in experimental fibres could have been contributed by synthesis of embryonic myosin induced by denervation was excluded because the heavy chains of purified rat embryonic myosin show an electrophoretic mobility intermediate between those of heavy chains of adult fast and slow myosin, and closer to that of slow MHC (Fig. 2, lane 4) and, therefore, it cannot co-migrate with the additional band. These findings demonstrate that the phenotypic expression of myosin isoforms may differ along the length of these single muscle fibres and suggest that this localization is related to the presence of a specific neural input. Clearly this trophic influence may only be exerted over a rather short distance from the endplate.

It is not known what is the message transmitted from nerve to muscle, nor which mediators in the muscle cell activate gene expression. Electrical activity may be the main message transmitted by a nerve^{5,16}, but there may be certain chemical factors (trophic factors)^{17,18}, some of which appear to control specific electrical characteristics of the muscle surface membrane¹⁹. The pattern of electrical activity seems important because the trains of action potentials propagate all along the fibre and could induce formation of mediators that control in a coordinated manner the activation of either 'fast' or 'slow' genes in all nuclei of the fibre. Moreover, direct stimulation by short high-frequency bursts of impulses (fast pattern of stimulation) is more effective than more prolonged trains of low-frequency impulses (slow pattern) in restoring normal membrane properties after denervation²⁰. But the pattern of electrical activity cannot fully explain why the expression of fast genes is restricted to the region around the endplate.

Nonetheless, the localized synthesis of fast myosin in a slow fibre indicates that only a few nuclei are affected by the fast endplate, and that specific chemical mediators must be formed in the muscle cell, but near the endplate. A preferential localization of a muscle membrane antigen (5.1.H11) near the nucleus responsible for its production has been reported in muscle heterokaryons *in vitro*²¹. On the other hand, 'mosaic' skeletal muscle fibres from mouse chimaeras show no spatial phenotypic heterogeneity related to genotypically distinct myonuclei²². These findings are not inconsistent with our results, because in mosaic fibres genotypically distinct myonuclei are randomly distributed, whereas in our experimental model it is the cytoplasmic mediator(s) which have a limited range of activity.

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1. Close, R. I. *Physiol. Rev.* **52**, 129-197 (1972).
2. Barany, M. *J. gen. Physiol.* **50**, 197-218 (1967).
3. Dhoot, G. K. & Perry, S. V. *Nature* **278**, 714-718 (1979).
4. Buller, A. J., Eccles, J. C. & Eccles, R. M. *J. Physiol., Lond.* **150**, 417-439 (1960).
5. Gutmann, E. & Hanzlikova, V. *Physiol. Bohem.* **16**, 244-250 (1967).
6. Lomo, T. & Slater, C. R. *J. Physiol., Lond.* **275**, 391-402 (1978).
7. Frank, E., Jansen, J. K. S., Lomo, T. & Westgaard, R. H. *J. Physiol., Lond.* **247**, 725-743 (1975).
8. Salviati, G., Betto, R. & Danielli-Betto, D. *Biochem. J.* **207**, 261-272 (1982).
9. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
10. Salviati, G., Betto, R., Danielli-Betto, D. & Zeviani, M. *Biochem. J.* **224**, 215-225 (1984).
11. Kuffler, D. P., Thompson, W. & Jansen, J. K. S. *Proc. R. Soc. B208*, 189-222 (1980).
12. Frank, E., Gautvik, K. & Sommerschild, H. *Acta Physiol., Scand.* **95**, 66-76 (1976).
13. Carraro, U. & Catani, C. *Biochem. biophys. Res. Commun.* **116**, 793-802 (1983).
14. Gauthier, G. F., Burke, R. E., Lowey, S. & Hobbs, A. W. *J. Cell Biol.* **97**, 756-771 (1983).
15. Salmons, S. & Sreter, F. A. *Nature* **263**, 30-34 (1976).
16. Pette, D. & Schnez, U. *FEBS Lett.* **83**, 128-130 (1977).
17. Guth, L. *Physiol. Rev.* **48**, 645-687 (1968).
18. Gutmann, E. A. *Rev. Physiol.* **38**, 177-216 (1976).
19. McArdle, J. J. *Prog. Neurobiol.* **21**, 135-198 (1983).
20. Lomo, T. & Westgaard, R. H. *J. Physiol., Lond.* **252**, 603-626 (1975).
21. Pavlath, G. K. & Blau, H. M. *J. Cell Biol.* **102**, 124-130 (1986).
22. Frair, P. M. & Peterson, A. C. *Expl. Cell Res.* **145**, 167-178 (1983).
23. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
24. Szent-Gyorgyi, A. G. *J. biol. Chem.* **192**, 361-369 (1951).

The tight junction does not allow lipid molecules to diffuse from one epithelial cell to the next

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The tight junction (zonula occludens) links epithelial cells into a monolayer by forming a continuous belt of sealing contacts around the apex of each cell. They appear in thin sections as if they were 'fusions' between the apposed plasma membranes¹ and in freeze-fracture replicas as patterns of complementary strands and furrows². These images have led to the proposal that the core of the tight junction is formed by a hexagonal cylinder of lipids^{3,4}. In this model, the cytoplasmic leaflet of the apical and basolateral plasma membrane domains would be continuous, whereas the exoplasmic leaflets of the two plasma membrane domains of the same cell would be separated at the tight junction and are instead predicted to be continuous between the plasma membranes of neighbouring cells. We demonstrate here that this prediction does not hold true. An endogenous glycolipid (Forssman antigen), present in the exoplasmic leaflet of the apical membrane of MDCK strain II cells^{5,6}, is unable to pass to MDCK strain I cells (which lack this glycolipid) under conditions where these cells are connected by tight junctions. In addition, fluorescent lipids which have been fused into the plasma membrane^{7,8} of one MDCK cell do not diffuse to neighbouring cells while the tight junctions between the cells are intact.

To test whether lipids can diffuse from one epithelial cell to another through continuous exoplasmic leaflets of their apical plasma membranes, we studied the behaviour of endogenous glycolipids. Strain II MDCK cells, a subline of the MDCK cell line⁹, possess a series of glycolipids, the globo series, which are not found in MDCK strain I cells, a different MDCK subline^{5,6}. Forssman antigen, one glycolipid of this series [GalNAc(α 1-3)GalNAc(β 1-3)Gal(α 1-4)Gal(β 1-4)Glc(β 1-1)Cer], constitutes 21% of the total neutral glycosphingolipids of MDCK strain II cells⁵. When a monolayer of these cells was labelled

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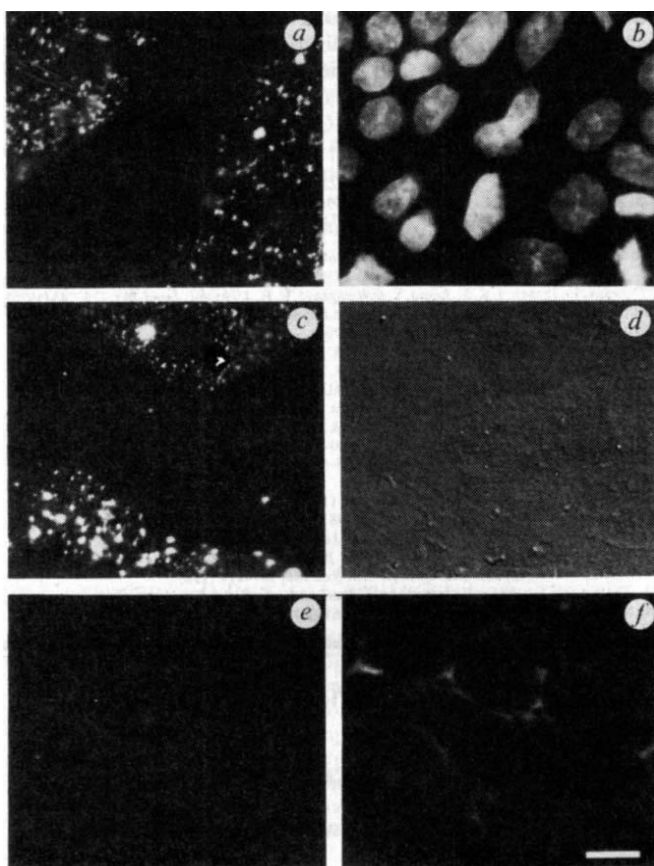


Fig. 1 Forssman antigen does not move from MDCK strain II cells to MDCK strain I cells during 96 h of co-culture. *a*, A mixed monolayer of MDCK strain I and strain II cells on nitrocellulose filters was fixed with 3% formaldehyde and incubated on the apical surface with monoclonal IgGs against Forssman antigen. Clustering of the fluorescent anti-Forssman antibodies in Fig. 1*a* is prevented if unfixed cells are incubated with the antibody at 0 °C and postfixed before addition of the second antibody (C. M. B. Butor and J. Davoust, unpublished results). As a second antibody we used rhodamine anti-mouse IgG. *b*, The same field of cells after staining the DNA in the nuclei by Hoechst dye 33258. *c*, A mixed monolayer grown on a glass coverslip stained apically against Forssman antigen as described for *a*. *d*, Nomarski optics micrograph of the field shown in *c*. *e*, Parallel monolayers on coverslips were incubated on the apical surface with a monoclonal antibody against uvomorulin, a lateral membrane marker in these cells¹⁰. As a second antibody we used rhodamine anti-mouse IgG. *f*, Staining with the anti-uvomorulin antibody after opening of the junctions by incubation with 2 mM EGTA for 5 min at 37 °C (ref. 10). Scale bar, 10 μm.

from the apical side with a monoclonal antibody against Forssman antigen, apical staining was observed (see also Fig. 4 of ref. 5). This glycolipid was therefore present in the exoplasmic leaflet of the apical plasma membrane. We co-cultured MDCK strain II cells with MDCK strain I cells which do not express Forssman antigen. The presence of intact tight junctions between the two cell types was demonstrated by adding a monoclonal antibody which recognizes uvomorulin, a protein present on the lateral membrane of MDCK cells¹⁰, and showing that it did not label the lateral membrane unless the tight junctions had been opened by treatment with 2 mM EGTA for 5 min at 37 °C before fixation (Fig. 1). Therefore, tight junctions were present between strain I and II cells grown together on nitrocellulose filters or glass slides. Staining with 33B12, a monoclonal IgG2c against Forssman antigen¹¹, demonstrated Forssman antigen on the apical surface of about 50% of the cells in the mixed monolayer under both growth conditions. The boundary between stained and unstained cells was sharp. Thus, the endogenous glycolipid

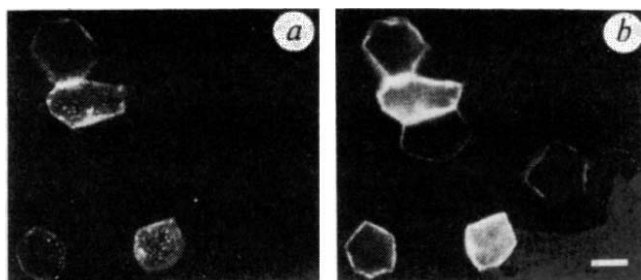


Fig. 2 The fluorescent phospholipid *N*-rhodamine phosphatidylethanolamine (N-Rh-PE) does not diffuse to neighbouring cells in a monolayer of MDCK strain I cells grown on nitrocellulose filters. N-Rh-PE, a water-insoluble lipid¹², was fused into the apical plasma membrane^{7,8} using unilamellar liposomes containing the fluorescent lipid equally distributed over both bilayer leaflets¹⁶. The cells were infected with 4 plaque-forming units per cell of influenza N virus and infection allowed to proceed for 4 h at 37 °C. The liposomes were prepared by reverse-phase evaporation¹⁹ from egg phosphatidylcholine, egg phosphatidylethanolamine, cholesterol, ganglioside G_{D1a} and N-Rh-PE (25:25:50:5:1, mol/mol). 20 nmol total lipid was added in 500 μl of serum-free medium to a monolayer of 3×10^6 cells. After 30 min at 0 °C, fusion was initiated by treatment with a serum-free medium buffered at pH 5.0 by 20 mM succinate, at 37 °C for 60 s. The cells were then returned to pH 7.4 medium and put on ice. Treatment of the cell surface with trypsin, necessary to cleave the haemagglutinin to its fusogenic form^{7,8}, was omitted because in strain I MDCK cells the haemagglutinin is cleaved spontaneously by a secreted protease (G.vanM., unpublished observations). Electrical resistance before infection was $6 \pm 2 \times 10^3 \Omega \text{ cm}^2$ ($n=3$), and after the complete procedure it was $1.2 \pm 0.2 \times 10^3 \Omega \text{ cm}^2$ ($n=6$). The microscope, equipped with a water immersion objective, was focused at the apical surface of the living cells (*a*) or halfway down the lateral surface (*b*), after liposome-cell fusion. Scale bar, 10 μm.

could not pass from MDCK strain II cells to the apical surface of neighbouring MDCK strain I cells over a period of 96 h of co-culture at 37 °C.

As an alternative approach, we fused^{7,8} fluorescent lipids into the apical plasma membrane of 50% or less of MDCK cells in a confluent monolayer and used a fluorescence microscope to observe whether the fluorescent lipid would move to non-fluorescent neighbour cells. For this, the fluorescent lipids dioleoyl *N*-rhodamine phosphatidylethanolamine (N-Rh-PE) or octadecyl rhodamine B (R18), which do not spontaneously exchange through the aqueous phase^{12,13}, were incorporated into liposomes. These liposomes were then added at 0 °C to the apical surface of MDCK cells infected with influenza virus. They were bound to the viral haemagglutinin glycoprotein expressed on the apical surface of these cells by including a haemagglutinin receptor into the liposomal membrane, the G_{D1a} ganglioside. Fusion was induced by a short treatment (60 s) at low pH (5.0) and 37 °C^{7,8}, after which the medium was readjusted to pH 7.4 and 0 °C. Lipid insertion was limited to a fraction of the cells in the epithelial monolayer by infecting the cells with a low multiplicity of influenza virus; in this way, only part of the cells in the monolayer became infected. Only the infected cells subsequently bound and fused liposomes.

MDCK strain I cells were selected for one set of experiments because they develop monolayers possessing a high electrical resistance ($>2,000 \Omega \text{ cm}^2$) when grown on a permeable support¹⁴, and this resistance can be used to assay the intactness of the tight junctions during the experiment^{10,15}. The fluorescent phospholipid N-Rh-PE was fused into the apical plasma membrane of about 50% of the MDCK strain I cells in a confluent monolayer (Fig. 2). Fusion was evident from the fact that the fluorescent phospholipid reached the basolateral surface (Fig. 2*b*). This process will be demonstrated and discussed in more detail elsewhere¹⁶. Fusion was also assayed by the hydrolysis of liposomal cholesterol oleate by a cellular enzyme⁸. The

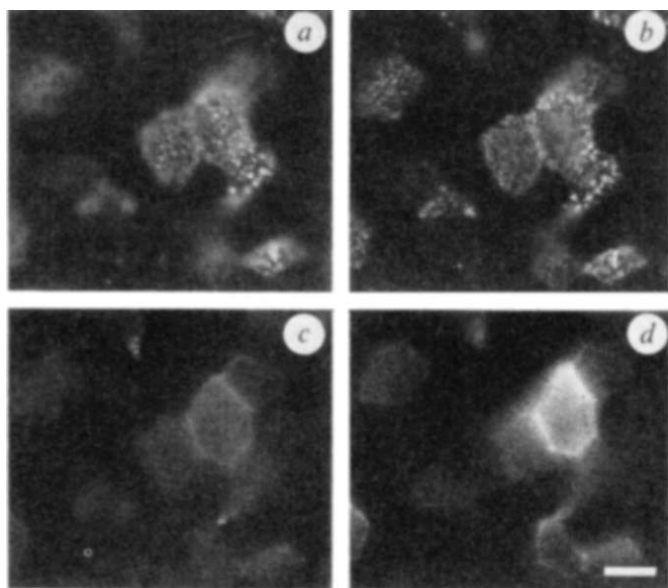


Fig. 3 The fluorescent lipid probe octadecyl rhodamine B (R18) does not pass from cell to cell in a monolayer of MDCK strain II cells on nitrocellulose filters. R18, a positively charged rhodamine lipid unable to exchange spontaneously between membranes¹³, was fused into the apical plasma membrane as in Fig. 2 with the following differences. At 5 h after infection with 4 plaque-forming units per cell of influenza N virus, the cell monolayer was treated with trypsin as described elsewhere^{7,8}. Large unilamellar liposomes were prepared by octyl β -D-glucoside dialysis from the same lipids as used in Fig. 2 but containing 1 mol % R18 instead of N-Rh-PE. The microscope was focused from the apical surface (a) down the lateral surface (b-d). The contorted orthogonal morphology of the columnar MDCK cells on nitrocellulose filters is clearly visible. Scale bar, 10 μ m.

electrical resistance of the monolayer was $1,200 \pm 160 \Omega \text{ cm}^2$ ($n = 6$) after the experiment, indicating that the tight junctions between the cells were essentially intact. In comparison, the electrical resistance across an intact polarized monolayer of strain II MDCK cells without virus infection was $160 \Omega \text{ cm}^2$. Under these circumstances, no spreading of the fluorescent phospholipid to the adjacent non-infected cells was observed. Bright and dark cells remained juxtaposed in the cell monolayer for hours.

Similar experiments were performed on low-resistance MDCK strain II cells using another lipid probe (Fig. 3). The fluorescent lipid R18 was fused into the apical plasma membrane. R18 differs from N-Rh-PE in that it carries one positive charge at neutral pH. As a criterion for intactness of the tight junction, the impermeability of the tight junction to antibodies was monitored as described above. The antibody rr1 against the lateral protein uvomorulin¹⁰ was unable to label its antigen throughout the experiment. Like N-Rh-PE, R18 remained confined to the cells into which it had been fused (Fig. 3a-d). No spreading from bright to dark cells was observed for hours at 0 °C.

We have presented evidence that three different types of lipids present in the apical plasma membrane domain are unable to diffuse into the apical membrane of adjacent cells. Dragsten *et al.*¹⁷ earlier observed that after partitioning water-soluble fluorescent probes into a monolayer of epithelial cells and bleaching one cell completely, no fluorescence returned into this black cell from the surrounding bright cells. In our experiments we used endogenous glycolipids and water-insoluble probes introduced by fusion, which we know are present in the outer leaflet of the apical membrane. We avoided the potentially damaging effects of photobleaching by observing mixed populations of cells containing and lacking the lipid studied. Finally, we also demonstrated that the tight junctions between relevant

cells were intact according to two criteria, both the trans-epithelial electrical resistance and the impermeability of the cell monolayer to antibodies. Moreover, we have tested the behaviour of the lipids at both 37 °C and 0 °C. Our observations argue against a continuity between the external leaflets of the apical plasma membrane in adjacent epithelial cells. The results are thus difficult to reconcile with the hexagonal lipid model of tight junction structure where the outer leaflets of the plasma membranes of neighbouring cells are fused^{3,4}. It seems more likely that proteins which are part of the tight junction structure^{10,18} bring the plasma membranes of adjacent cells very close together but do not induce a partial fusion. This would also be more consistent with the fact that the tight junction, as a barrier to ion diffusion between cells, displays ion selectivity¹⁵.

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1. Farquhar, M. G. & Palade, G. E. *J. Cell Biol.* **17**, 375-412 (1963).
2. Goodenough, D. A. & Revel, J. P. *J. Cell Biol.* **45**, 272-290 (1970).
3. Kachar, B. & Reese, T. S. *Nature* **296**, 464-466 (1982).
4. Pinto da Silva, P. & Kachar, B. *Cell* **28**, 441-450 (1982).
5. Hansson, G. C., Simons, K. & van Meer, G. *EMBO J.* **5**, 483-489 (1986).
6. Nichols, G. E., Lovejoy, J. C., Borgman, C. A. & Young, W. W. Jr *J. Cell Biol.* **101**, 64a (1985).
7. van Meer, G. & Simons, K. *J. Cell Biol.* **97**, 1365-1374 (1983).
8. van Meer, G., Davoust, J. & Simons, K. *Biochemistry* **24**, 3593-3602 (1985).
9. Balcarova-Ständer, J., Pfeiffer, S. E., Fuller, S. D. & Simons, K. *EMBO J.* **3**, 2687-2694 (1984).
10. Gumbiner, B. & Simons, K. *J. Cell Biol.* **102**, 457-468 (1986).
11. Sonnenberg, A. *et al. J. Immun.* (in the press).
12. Struck, D. K., Hoekstra, D. & Pagano, R. E. *Biochemistry* **20**, 4093-4099 (1981).
13. Hoekstra, D., de Boer, T., Klappe, K. & Wilschut, J. *Biochemistry* **23**, 5675-5681 (1984).
14. Fuller, S., von Bonsdorff, C.-H. & Simons, K. *Cell* **38**, 65-77 (1984).
15. Cerejido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A. & Sabatini, D. D. *J. Cell Biol.* **77**, 853-880 (1978).
16. van Meer, G. & Simons, K. *EMBO J.* **5**, 1455-1464 (1986).
17. Dragsten, P. R., Blumenthal, R. & Handler, J. S. *Nature* **294**, 718-722 (1981).
18. Stevenson, B. R. & Goodenough, D. A. *J. Cell Biol.* **98**, 1209-1221 (1984).
19. Szoka, F. Jr & Papahadjopoulos, D. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4194-4198 (1978).

Synthetic peptides as nuclear localization signals

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The nuclear envelope defines a compartment boundary which is penetrated by pores that mediate a remarkable transport process. Precursor RNAs are retained in the nucleus, while processed messenger RNA¹, transfer RNA² and ribosomal subunits³ are transported to the cytoplasm. Proteins destined for the nucleus become localized soon after synthesis and again following mitosis, while cytoplasmic proteins are excluded⁴. The process is highly specific: a single base change in vertebrate initiator tRNA^{Met} (tRNA^{iMet}) reduces the rate of export 20-fold⁵; a point mutation within the simian virus 40 (SV40) large-T antigen, converting Lys 128 to Thr (ref. 6) or Asn (ref. 7), prevents import. Lys 128 lies within a short 'signal' sequence which, when fused to large non-nuclear proteins, causes their accumulation in nuclei⁶⁻⁸. Regions of other eukaryotic proteins also seem to contain nuclear localization signals, although a single consensus sequence has not emerged⁹⁻¹³. We report here that a synthetic peptide containing 10 residues of large-T antigen sequence serves as a nuclear localization signal when cross-linked to bovine serum albumin (BSA) or immunoglobulin G (IgG) and microinjected in *Xenopus* oocytes. Substitution of Thr at the position of Lys 128 in this peptide

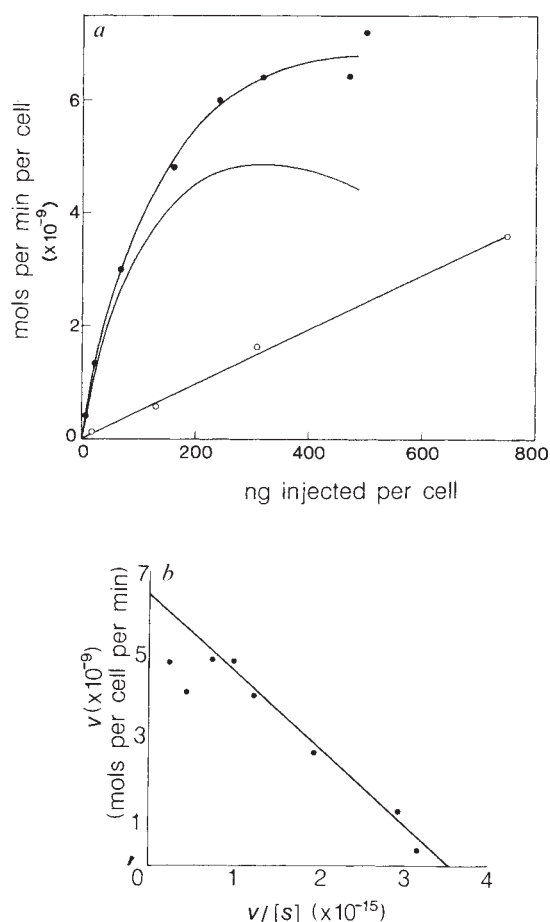


Fig. 2 *a*, Initial rate of nuclear accumulation of P(Lys)-BSA as a function of amount injected. ●, P(Lys)-BSA; ○, BSA. Each initial rate was determined from measurements on at least two groups of six to eight oocytes, injected with ^{125}I -P(Lys)-BSA or ^{125}I -BSA, incubated for 10–45 min at 25 °C, and processed as for Fig. 1. The central curve (no data points) represents the difference between the two experimentally determined curves. *b*, Eadie-Hofstee plot of data from *a*. Reaction velocities (v) were derived from the initial rate data for P(Lys)-BSA by subtraction of values from the curve for BSA. Concentrations ($[S]$) of P(Lys)-BSA in oocytes were calculated as described for Table 2.

sections (data not shown). P(Lys)-BSA isolated from nuclei was indistinguishable in size from starting material (Fig. 1*b*) and therefore had not entered as proteolytic fragments. We conclude that the attachment of signal peptides to the surface of proteins too large to enter the nucleus by diffusion is sufficient to effect their nuclear localization, and moreover, that the process is signal-sequence-specific, with no simple dependence on amino-acid composition (for example, on net positive charge^{17,18}).

The nuclear accumulation of P(Lys)-BSA showed saturation kinetics (Fig. 2*a*), when corrected for entry into the nucleus by diffusion¹⁹, which became appreciable when large amounts of material were injected. (Rates of entry by diffusion were determined with unmodified BSA, and may represent slight overestimates for P(Lys)-BSA, which is expected to enter more slowly due to its larger size.) An Eadie-Hofstee plot was linear (Fig. 2*b*), with intercepts corresponding to a K_M of 1.8×10^{-6} M and $V_{\max}$ of 6.4×10^9 molecules per cell per min. (Rates of nuclear accumulation varied, depending on the batch of oocytes and extent of iodination of the protein, so the values of K_M and $V_{\max}$ should be regarded as approximate.) Deviations from linearity at the two highest values of substrate concentration probably reflect the inaccuracy of the correction for diffusion.

The peptide-linked proteins used in this work differ from natural substrates for nuclear transport in possessing multiple signal sequences, and the question arises of whether a single peptide would be effective. We investigated this question by co-injecting P(Lys)-BSA with excess free peptide (Table 2).

Table 2 Effect of free peptide on nuclear accumulation of peptide-linked BSA

Free peptide	Expt 1	Expt 2
None	23.6 ± 8.2	27.1 ± 11.1
P(Lys)	10.9 ± 3.4 (0.01 < P < 0.02)	11.3 ± 4.1 (0.02 < P < 0.05)
P(Thr)	19.0 ± 4.4 (0.02 < P < 0.05)	19.0 ± 6.9 (0.05 < P < 0.1)
P(H2B)	—	25.1 ± 8.7 (0.2 < P < 0.5)

Approximately 5 ng of ^{125}I -P(Lys)-BSA (6×10^{-5} nmol) was injected alone or with the free peptide (0.5 nmol) indicated in 16–18 oocytes. Approximate concentrations of P(Lys)-BSA and free peptide in an oocyte were 1×10^{-7} and 0.9×10^{-3} M, respectively, based on an accessible aqueous volume of 0.5 μl (one-third)¹⁶ of a total volume of 1.5 μl plus an average volume injected of 60 nl. Following incubation for 40 min at 18 °C, ^{125}I in nucleus and cytoplasm were determined as in Fig. 1. Data are expressed as the per cent of the total counts found in the nucleus. The probability (P) that added peptide did not affect uptake was determined with a two-tailed t -test.

Nuclear accumulation of the peptide-linked protein was markedly reduced by free P(Lys) and to a lesser extent by P(Thr). Only the rate and not the final extent of nuclear accumulation was affected (data not shown). Co-injection of P(H2B) caused no significant reduction in rate, arguing against indirect effects of the peptide preparations. We conclude that free peptide interacts specifically with a component of the nuclear transport apparatus, although the affinity of the interaction is low, as 1 mM peptide is required for ~50% inhibition of transport. The difference between the micromolar K_M for P(Lys)-BSA and the much lower affinity for free peptide may reflect a multivalent interaction of the peptide-linked protein (see ref. 18), but further work is needed to explain the discrepancy.

The saturation kinetics and effect of free peptide on nuclear transport are indicative of a limiting component of the uptake apparatus. This component may be a receptor for the signal peptide or it may interact with a receptor-signal peptide complex. The receptor may be diffusible, as in the case of 'signal recognition particle' in the synthesis of membrane and secreted proteins²⁰, or it may reside in the nuclear pore complex. Signal peptide may interact not only with this receptor but also with factors inside the nucleus. Thus, the apparent capacity of P(Thr)-linked proteins to enter but not accumulate in the nucleus may be explained either by their altered interaction with the receptor or by their lack of retention (for example, through binding) in the nucleus. Further modifications of the signal peptide sequence and kinetic analyses may help to distinguish between these possibilities.

A nuclear uptake receptor would presumably be general, recognizing signal sequences not only in large-T antigen but also in other nuclear proteins. We have used anti-signal peptide antibodies to investigate the occurrence of large-T-like sequences in other proteins. Antisera against P(Lys) reacted strongly with several nuclear proteins from human lymphocytes (Fig. 3). This strong reaction was abolished by pre-incubation of antisera with P(Lys) (two weakly reacting components at relative molecular mass (M_r) ~45,000 (45K) and 65K persisted). There was only a weak reaction with a small number of cytoplasmic proteins, and these appeared to correspond with nuclear proteins, which may have leaked from nuclei during isolation. These data are indicative of a family of proteins bearing homologous nuclear localization signals and sharing a common receptor. Conceivably, all nuclear proteins share the same receptor, whose

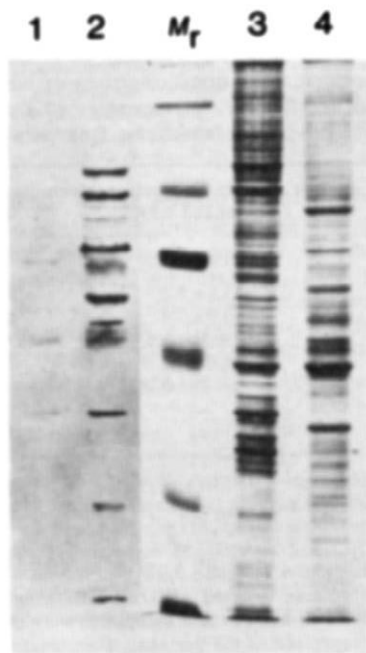


Fig. 3 Reaction of antiserum against P(Lys) with proteins from human lymphocytes. A SDS-10% polyacrylamide gel of cytoplasmic (lanes 1, 4) and nuclear (lanes 2, 3) extracts is shown before (lanes 3, 4, stained with Coomassie blue) and after (lanes 1, 2) immunoblotting. M_r of markers from the top of the gel, are 200 K, 97.4 K, 68 K, 43 K, 25.7 K, 18.4 K.

Methods. MICH cells (an Epstein-Barr virus-immortalized human B-lymphocyte line, from Dr P. Parham, Stanford University) were grown in RPMI-1640 supplemented with 10% fetal bovine serum. Cells (6×10^6) were washed with cold phosphate-buffered saline and lysed by suspension in 14 ml of 0.34 M sucrose, 10 mM Tris-HCl pH 7.8, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% Nonidet-P40, 10 mM β -mercaptoethanol, 0.5 mM phenylmethanesulphonyl fluoride (PMSF). One ml of the lysate was centrifuged (Eppendorf) for 5 min, and the supernatant was retained as cytoplasmic extract. To the remaining lysate was added 14 ml of buffer B (2 M sucrose, 10 mM Tris-HCl pH 7.8, 5 mM $MgCl_2$, 10 mM β -mercaptoethanol, 0.5 mM PMSF), and the mixture was layered over 3 ml of buffer B and centrifuged for 50 min at 25,000 r.p.m. in a Beckman SW41 rotor. The pellet was suspended in 3 ml of 50 mM Tris-HCl pH 7.8, 0.1 M NaCl, 10 mM β -mercaptoethanol, 0.5 mM PMSF, and 2 ml of 2.5 M KCl was added to give nuclear extract. The cytoplasmic and nuclear extracts were centrifuged for 30 min at 50,000 r.p.m. in a Beckman TL-100 ultracentrifuge. Aliquots (30 μ g of protein) of the supernatants were fractionated in polyacrylamide gels and electroblotted onto a nitrocellulose filter, and the filter was washed in 0.9% NaCl, 10 mM Na-phosphate, 0.05% Tween-20 pH 7.4 and incubated for 1 h with anti-P(Lys) antiserum (1:500 dilution). Antibody bound to the filter was detected with the Vectastain ABC horseradish peroxidase system as described by the manufacturer (Vector). Anti-P(Lys) antiserum was obtained from rabbits 10 days after a second injection with 200 μ g of P(Lys)-thyroglobulin (prepared as for Fig. 1 with bovine thyroglobulin type 1 (Sigma), injected first with Freund's complete adjuvant and 14 days later with incomplete adjuvant).

specificity is broader than that of the anti-peptide antibodies used here. Alternatively, there may be additional receptors for other classes of nuclear protein bearing different signal sequences.

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- Wickens, M. P. & Gurdon, J. B. *J. molec. Biol.* **163**, 1-26 (1983).
- Melton, D. A., DeRobertis, E. M. & Cortese, R. *Nature* **284**, 143-148 (1980).
- Warner, J. R., Tushinski, R. J. & Wejksnora, P. J. in *Ribosomes: Structure, Function and Genetics* (eds Chambiss, G. et al.) 889-902 (University Park Press, Baltimore, 1980).
- Bonner, W. M. in *The Cell Nucleus* Vol. 6C (ed. Busch, H.) 97-148 (Academic, New York, 1978).
- Zaslloff, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6436-6440 (1983).
- Kalderon, D., Richardson, W. D., Markham, A. F. & Smith, A. E. *Nature* **311**, 33-38 (1984).
- Lanford, R. E. & Butel, J. S. *Cell* **37**, 801-813 (1984).
- Kalderon, D., Roberts, B. L., Richardson, W. D. & Smith, A. E. *Cell* **39**, 499-509 (1984).
- Dingwall, C., Sharnick, S. V. & Laskey, R. A. *Cell* **30**, 449-458 (1982).
- Silver, P. A., Keegan, L. P. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5951-5955 (1984).
- Hall, M. N., Hereford, L. & Hershowitz, I. *Cell* **36**, 1057-1065 (1984).
- Moreland, R. B., Nam, H. G., Hereford, L. M. & Fried, H. M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6561-6565 (1985).
- Davey, J., Dimmock, N. J. & Colman, A. *Cell* **40**, 667-675 (1985).
- Van Helden, P., Strickland, W. N., Brand, W. F. & Von Holst, C. *Biochim. biophys. Acta* **533**, 278-281 (1978).
- Michaeli, T. & Prives, C. *Molec. cell. Biol.* **5**, 2019-2028 (1985).
- Bonner, W. M. *J. Cell Biol.* **64**, 421-430 (1975).
- Smith, A. E. et al. *Proc. R. Soc. B226*, 43-58 (1985).
- Richardson, W. D., Roberts, B. L. & Smith, A. E. *Cell* **44**, 77-85 (1980).
- Paine, P. L., Moore, J. R. & Horowitz, S. D. *Nature* **254**, 104-114 (1975).
- Wickner, W. T. & Lodish, H. F. *Science* **230**, 400-407 (1985).
- Laemmli, V. K. *Nature* **227**, 680-685 (1970).
- Erickson, B. W. & Merrifield, R. B. in *The Proteins* Vol. 2, 3rd edn (eds Neurath, H., Hill, R. L. & Boeda, C.-L.) 257-493 (Academic, New York, 1976).
- Watts, T. H., Gariépy, J., Schoolnik, G. K. & McConnell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5480-5484 (1985).
- Peltz, G., Spudich, J. A. & Parham, P. *J. Cell Biol.* **100**, 1016-1023 (1985).
- Dulbecco, R. & Vogt, M. *J. exp. Med.* **99**, 167 (1954).
- Gurdon, J. B. *J. Embryol. exp. Morph.* **36**, 523-540 (1976).
- Bradford, M. *Analyt. Biochem.* **72**, 248-254 (1976).

Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma

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The application of recombinant DNA techniques has identified two fundamental mechanisms of tumorigenesis in man. The first involves a qualitative or quantitative change in an oncogene (see ref. 1 for review). In the second, discovered in embryonal tumours, a primary mutation occurs which is recessive at the cellular level to the normal allele. The growth of a tumour ensues only after a secondary change, such as chromosome loss or mitotic recombination, eliminates the normal allele, thereby unmasking the altered allele²⁻⁷. Because its effect is recessive, the primary mutation may also occur and be transmitted in the germ line, resulting in a familial pattern for the disease. In familial cases, independent bilateral tumours are common, since the tumours result from a single event—loss of the normal genes—which can occur in any cell. This contrasts with non-familial (sporadic) cases where solitary tumours result from the infrequent occurrence of two rare events within the same cell²⁻⁷. By a molecular genetic approach we have now shown that acoustic neuroma, one of the most common tumours of the human nervous system⁸, is specifically associated with loss of genes on human chromosome 22 and may result from the mechanism of tumorigenesis discovered in embryonal tumours. This finding might provide a clue to the chromosomal location of the defective gene in bilateral acoustic neurofibromatosis, an autosomal dominant disorder with the hallmark of bilateral acoustic neuromas⁹⁻¹². In view of the frequent occurrence of meningiomas in patients with bilateral acoustic neurofibromatosis and the association of meningioma with loss of chromosome 22 previously reported in cytogenetic studies¹³⁻¹⁵, we suggest that a common event underlies tumorigenesis in acoustic neuroma and meningioma.

Table 1 Loss of heterozygosity on chromosome 22 in acoustic neuromas

Marker Enzyme Chr	<i>SIS</i> <i>Hin3</i> 22	<i>D22S1</i> <i>BglII</i> 22	<i>IGLC</i> <i>EcoRI</i> 22	<i>NGFB</i> <i>BglII</i> 1	<i>NGFB</i> <i>TaqI</i> 1	<i>D4S10</i> <i>HinIII</i> 4	<i>D4S35</i> <i>MspI</i> 4	<i>D10S1</i> <i>TaqI</i> 10	<i>HRAS1</i> <i>TaqI</i> 11	<i>D11S17</i> <i>MspI</i> 11	<i>D11S17</i> <i>BglII</i> 11
Patient											
AN1	12	12	—	—	—	—	—	—	—	12	—
AN2	12	12	12	—	—	—	—	—	12	12	12
AN3	—	2	—	—	12	12	—	—	—	12	—
AN4	—	—	—	—	—	12	—	—	12	12	12
AN5	1	1	—	—	12	—	—	—	—	12	—
AN6	—	12	—	—	—	—	12	—	12	12	12
AN7	1	—	—	12	—	—	—	—	12	—	12
AN8	—	—	—	12	—	—	—	—	12	—	—
AN9	—	—	—	12	—	12	—	—	—	—	—
AN10	12	—	—	—	—	—	12	—	—	—	—
AN11	—	2	—	—	—	12	—	—	12	—	12
AN12	—	—	1	12	12	—	—	—	—	—	—
AN13	2	—	—	—	12	12	12	—	—	—	—
AN14	—	2	—	—	—	—	—	12	—	—	—
AN15	—	12	12	—	—	—	—	—	12	—	—
AN16	—	12	—	—	—	12	—	—	12	—	—
AN17	—	12	—	—	12	—	—	—	12	—	12
AN18	—	12	—	—	—	12	—	—	—	—	12
AN19	—	—	—	—	—	—	—	—	—	—	—
AN20	—	—	—	—	—	12	—	12	—	—	12
AN21	—	12	12	12	—	12	—	12	12	—	—

Marker Enzyme Chr	<i>INS</i> <i>SacI</i> 11	<i>KRAS2</i> <i>TaqI</i> 12	<i>D13S1</i> <i>MspI</i> 13	<i>D13S4</i> <i>MspI</i> 13	<i>D13S5</i> <i>EcoRI</i> 13	<i>D13S7</i> <i>BglII</i> 13	<i>D14S1</i> <i>EcoRI</i> 14	<i>GH</i> <i>MspI</i> 17	<i>D18S3</i> <i>MspI</i> 18	<i>C3</i> <i>HinIII</i> 19	<i>C3</i> <i>SacI</i> 19	<i>D21S17</i> <i>BglII</i> 21
Patient												
AN1	12	—	12	12	—	—	12	12	12	—	—	—
AN2	—	—	12	—	—	—	12	12	—	—	—	12
AN3	12	12	12	12	—	—	—	12	12	—	—	12
AN4	12	—	12	—	—	—	—	12	—	—	12	12
AN5	—	—	12	—	12	—	—	12	—	—	—	12
AN6	12	12	12	12	—	—	—	—	12	12	—	12
AN7	—	12	—	12	—	—	12	12	—	—	—	—
AN8	—	—	—	—	—	—	12	12	—	—	12	—
AN9	—	—	—	—	—	12	12	—	12	—	—	12
AN10	—	—	—	12	12	—	—	—	12	—	—	—
AN11	—	—	—	—	12	—	12	—	—	—	—	12
AN12	—	—	—	—	—	—	12	—	—	—	—	—
AN13	—	—	—	12	12	—	—	12	12	—	—	—
AN14	—	12	—	—	12	—	12	—	—	—	12	12
AN15	—	—	—	12	—	—	—	12	—	12	12	—
AN16	—	—	—	—	—	—	—	—	—	—	—	—
AN17	—	—	—	—	—	—	12	12	12	—	12	—
AN18	—	—	—	12	—	—	—	—	—	—	—	—
AN19	—	—	—	—	—	—	12	—	12	—	—	12
AN20	—	12	—	—	—	—	—	—	—	—	12	12
AN21	—	—	—	12	12	12	12	—	12	—	12	12

Chr, chromosome. Tumour and normal DNA from acoustic neuroma patients was digested with the indicated restriction enzymes, fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized to probes for the indicated loci as described in Fig. 1. In addition to the three chromosome 22 probes listed in Fig. 1, the following probes from other chromosomes were used: N8C6 (*NGFB*)^{21,22}, G8 and R7 (*D4S10*)^{23,24}, G9 (*D4S35*)²⁵, Dry5-1 (*D10S1*)²⁶, pEJ6.6 (*HRAS1*)²⁷, pHINS321 (*INS*)²⁸, pJ19.4 (*D11S17*)²⁶, p640 (*KRAS2*)²⁷, p7F12 (*D13S1*)²⁹, p1E8 (*D13S4*)²⁹, pHUB8 (*D13S5*)³⁰, pHU26 (*D13S7*)³⁰, pAW101 (*D14S1*)³¹, C-H800 (*GH*)³², B74 (*D18S3*)²⁶, pC3 (*C3*)³³ and pGSH8 (*D21S17*)³⁴. These probes are known to reveal RFLPs in human genomic DNA digested with the indicated restriction enzymes. The phenotype observed in the tumour DNA is shown for every case where the blood DNA displayed heterozygosity: '12' indicates heterozygosity (even though different pairs of alleles may be present for certain multi-allele markers), '1' indicates continued presence of the larger allelic restriction fragment and loss of the smaller allelic fragment relative to normal tissue DNA, and '2' indicates continued presence of the smaller allelic restriction fragment and loss of the larger fragment. Where the normal DNA was tested but was uninformative because it did not display heterozygosity a '-' has been entered to simplify consideration of the data. The absence of an entry indicates that a marker was not tested or did not give a readable result for that particular patient.

Acoustic neuroma (also called vestibular schwannoma or acoustic neurinoma) derives from Schwann cells enveloping the vestibular branch of the vestibulocochlear nerve¹⁶. The majority of acoustic neuromas occur as solitary non-inherited unilateral tumours. By contrast, bilateral tumours are the hallmark of the familial cases of acoustic neuroma that characterize central or 'bilateral acoustic' neurofibromatosis⁹⁻¹². The differences in presentation of sporadic and familial cases of acoustic neuroma are reminiscent of retinoblastoma and Wilms' tumour, two cancers for which loss of a particular chromosomal region (13q and 11p respectively) has been implicated in tumour development²⁻⁷.

We therefore used polymorphic DNA markers to search for similar loss of particular chromosome regions in acoustic neuroma. DNA was isolated from primary acoustic neuromas

and corresponding leukocytes from 21 patients (5 male, 16 female). The frequency of both meningiomas and acoustic neuromas in bilateral acoustic neurofibromatosis, and the loss of chromosome 22 previously reported in cytogenetic investigations of meningioma¹³⁻¹⁵ prompted us to focus our initial efforts on DNA markers for chromosome 22. We typed genomic DNA with three polymorphic chromosome 22 markers: *SIS*, the platelet derived growth factor locus homologous to the *sis* oncogene mapping to 22q12.3-13.1; *D22S1*, an anonymous DNA locus detected by probe pMS3-18 mapping to 22pter-q13; and *IGLC*, the λ immunoglobulin constant region at 22q11. The results of this analysis are presented in Table 1.

Sixteen patients were heterozygous in their normal tissue for at least one of the three polymorphic DNA markers and were consequently informative for determining whether loss of

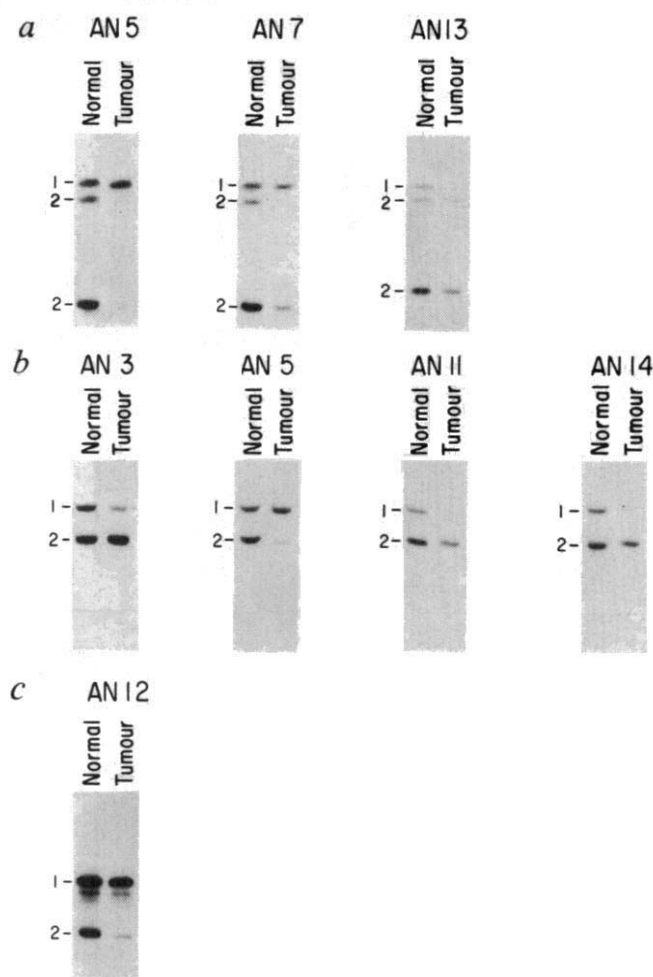


Fig. 1 Loss of constitutional heterozygosity of loci on human chromosome 22 in acoustic neuromas. Patient designations are shown above the autoradiograms. Numbers on the left indicate the observed alleles, with '1' and '2' referring to the larger and smaller allelic restriction fragments respectively. Southern blots were hybridized²² to ³²P-labelled DNA probes for the following loci on chromosome 22: *a*, *Psis.1* (*Oncor*) (*SIS* oncogene); *b*, *pMS3-18* (*D22S1*); *c*, *HuλC2* (*IGLC*). *Psis.1* reveals a RFLP with fragments of 21 kilobases (kb) ('1'-allele), and 14.5 and 6.5 kb ('2'-allele) in *Hind*III-digested human genomic DNA^{35,36}. The *pMS3-18* probe detects a RFLP in *Bgl*III-digested human DNA, with fragments of 9.5 kb ('1'-allele) and 6.5 kb ('2'-allele)³⁷. The *HuλC2* probe reveals a multi-allele RFLP represented by fragments of 8 kb, 13 kb, 18 kb and 23 kb in *Eco*RI-digested human genomic DNA²⁶. To simplify the nomenclature, the higher relative molecular mass allele is called the '1'-allele, whereas the lower relative molecular mass allele is termed the '2'-allele in each case. **Methods.** High relative molecular mass DNA was isolated from surgical tumour specimens and corresponding normal tissue (peripheral leukocytes). The tumour specimens which had been immediately frozen on dry ice after operation, were rapidly washed, rinsed and suspended overnight at 37 °C in a TNE-SDS-proteinase K solution (10 mM Tris, pH 8.0; 100 mM NaCl; 1 mM EDTA, 1% SDS; 200 μg ml⁻¹ proteinase K). Nuclei from peripheral leukocytes were isolated by gently homogenizing peripheral venous blood in a solution containing 0.64 M sucrose, 20 mM Tris, 10 mM MgCl₂, 5% (v/v) Triton X-100 pH 7.6 at 4 °C. The isolated nuclei were subsequently incubated overnight at 37 °C in the TNE-SDS-proteinase K-solution. DNA from tumour and leukocyte tissue samples was purified by phenol and chloroform extraction²³. Approximately 5 μg of DNA was digested to completion with the restriction enzymes *Hind*III, *Bgl*III or *Eco*RI (New England Biolabs). The resulting DNA fragments were fractionated by agarose gel electrophoresis and transferred to nylon membrane²³.

constitutional heterozygosity had occurred in their respective tumour tissue. Seven of the 16 informative cases (44%) showed loss or marked reduction in the intensity of one of the two alleles in their tumour tissue (Table 1). These cases are displayed in Fig. 1. When analysed by densitometry, the hybridization signals corresponding to the deleted allele were estimated to be diminished by 60% (patient AN3), 86% (AN5), 75% (AN7), 73% (AN11), 75% (AN12), >90% (AN13) and 78% (AN14), respectively. The remaining hybridization signals in the tumour DNAs might be due to contaminating non-tumour cells (vascular or connective tissue) which may be present in primary surgical specimens. Alternately, the acoustic neuromas might consist of a mosaic of cells with normal karyotype, and cells with monosomy for chromosome 22.

To distinguish among the different mitotic mechanisms that might have produced this loss of heterozygosity, the hybridization signals from Fig. 1 were compared with those obtained when the same filters were rehybridized with several probes for loci on other chromosomes. The results obtained are presented in Table 2. The ratio of the copy number of chromosome 22 between tumour and normal tissue was approximately 1:2 in all seven cases. This is indicative of 'true' loss of one copy of the chromosome 22 locus, rather than mitotic recombination, loss and reduplication, or gene conversion. Furthermore, patient AN5 was constitutionally heterozygous for two of the three chromosome 22 loci tested. Heterozygosity was lost at both loci in the corresponding tumour tissue, suggesting loss of the entire chromosome rather than a small partial deletion. None of these acoustic neuromas showed mitoses or other aggressive features upon pathological examination; neither did hemizygosity for

chromosome 22 show any striking correlation with the patient's age, sex, tumour size or clinical course.

Loss of genes on chromosome 22 was not restricted to unilateral sporadic cases of acoustic neuromas, but was also observed in a tumour from patient AN14 who had bilateral acoustic neuromas. By analogy to inherited cases of retinoblastoma and Wilms' tumour, the defect in bilateral acoustic neurofibromatosis could map to chromosome 22. Tumours from patients AN10 and AN18, two cases of bilateral acoustic neurofibromatosis, did not show loss of heterozygosity for chromosome 22 markers. It should be noted, however, that alternative mitotic or mutational events leading to expression of a recessive disease gene could occur in either sporadic or familial cases without apparent loss of heterozygosity for the available DNA markers. For example, neither a new mutation, a gene conversion, nor a mitotic recombination with a breakpoint distal to the three markers would be recognized. It is conceivable that one or more of these three mechanisms have occurred in the nine tumours for which heterozygosity of the chromosome 22 markers was maintained.

The chromosomal specificity of the loss of heterozygosity in acoustic neuromas was investigated with several polymorphic DNA markers for 11 other chromosomes (Table 1). In each case where constitutional heterozygosity was observed, heterozygosity was also maintained in the corresponding tumour tissue. Thus, acoustic neuromas exhibit a remarkable genome stability, with chromosomal losses being highly specific to chromosome 22. The data in Table 1 also provide assurance that the differences between tumour and normal DNA on chromosome 22 did not arise from simply mispairing of the samples or unreliability

Table 2 Quantitative densitometry of probe hybridization for acoustic neuromas losing heterozygosity on chromosome 22

Patient	Tissue	Chromosome 22	
		Control chromosome	Tumour Normal
AN3	Acoustic neuroma	0.58	0.50
	Leukocytes	1.17	
AN5	Acoustic neuroma	0.14	0.42
	Leukocytes	0.33	
AN7	Acoustic neuroma	0.71	0.52
	Leukocytes	1.35	
AN11	Acoustic neuroma	0.08	0.50
	Leukocytes	0.16	
AN12	Acoustic neuroma	1.48	0.43
	Leukocytes	3.46	
AN13	Acoustic neuroma	0.09	0.63
	Leukocytes	0.14	
AN14	Acoustic neuroma	0.09	0.51
	Leukocytes	0.17	

Densitometry of autoradiograms is subject to a number of variables. Therefore, to determine whether loss of one allele for chromosome 22 was associated with duplication of the remaining allele, it was necessary to control for these variations by normalizing the data for chromosome 22 probes to that obtained from the same blots using probes for other chromosomes. Southern blots which had been hybridized to probes for chromosome 22 (Fig. 1) were freed of these probes in distilled water for 2 h at 65 °C and rehybridized with probes for loci on other chromosomes ('Control chromosomes'): (1) *D4S10* on chromosome 4p for AN7 and AN13; (2) *D21S17* on chromosome 21q for AN3, AN5, AN11 and AN14; and (3) *D13S5* on chromosome 13q for AN12. Heterozygosity for RFLPs of these markers frequently provided clear confirmation that they were not deleted in the tumor DNA. The autoradiograms from all hybridizations were analysed by scanning densitometry with an LKB Ultrascan XL. The peak areas corresponding to each hybridization signal were calculated by electronic integration. In order to determine that the approach of normalizing the hybridization signals yielded reliable results, we first compared hybridization signals for pairs of markers from other chromosomes (selected from the markers used in Table 1) for which heterozygosity in the tumours was maintained. These invariably gave constant ratios for tumour and normal DNA. We then determined whether any chromosome 22 alleles had been duplicated in the tumours by normalizing hybridization signals specific to chromosome 22 relative to hybridization signals for control chromosome probes in the same sample, and then obtaining a ratio of the normalized values for each tumour/normal tissue pair.

of the applied techniques. Indeed, all data in Table 1 with probes detecting restriction fragment length polymorphisms (RFLPs) in *HindIII*, *BglII* and *EcoRI* digested DNA were obtained by rehybridizing the respective Southern blot filters already used for studies on chromosome 22.

The finding that acoustic neuromas specifically lose genes on chromosome 22 has several major implications. It extends the

concept that specific somatic loss of constitutional heterozygosity can be a mechanism of tumorigenesis by demonstrating that it can operate in neural crest-derived tumours of the adult nervous system. It suggests that a similar mechanism is involved in development and growth of acoustic neuromas and meningiomas since the latter are also associated with loss of chromosome 22¹³⁻¹⁵ and both tumours occur frequently in bilateral acoustic neurofibromatosis⁹⁻¹². Of particular interest, this result may lead to the chromosomal localization of the defect causing bilateral acoustic neurofibromatosis, a serious genetic disorder that can lead to deafness or other neurological morbidity or mortality within the first few decades of life⁹⁻¹². Retinoblastoma and Wilms' tumour have become models for the concept that chromosomal mechanisms may serve to unmask recessive mutant alleles by elimination of the wild-type gene²⁻⁷. Unilateral occurrence of acoustic neuromas in sporadic cases, and bilateral tumour formation as the hallmark of the inherited form (bilateral acoustic neurofibromatosis) agree well with this model. Thus, genes on chromosome 22 may play a part in acoustic neuroma analogous to that of genes on chromosomes 11 and 13 in Wilms' tumour and retinoblastoma, respectively. This would suggest that chromosome 22 contains a gene important for the development of acoustic neuromas that is transmitted in its defective form in bilateral acoustic neurofibromatosis.

The region of 11p associated with Wilms' tumour has more recently been tied to rhabdomyosarcoma and hepatoblastoma¹⁷. All three of these rare embryonal cancers cluster in the Beckwith-Wiedemann syndrome¹⁷. Similarly, the region of 13q implicated in retinoblastoma has now been associated with osteosarcoma¹⁸. The human genome may contain a limited number of such loci whose defective alleles predispose to tumour formation in particular tissues. The striking frequency of clinical association of acoustic neuromas and meningiomas in bilateral acoustic neurofibromatosis⁹⁻¹² and the observation that meningioma tissue can be adjacent to or within acoustic neuroma tissue^{19,20} suggests that the selective loss of genes on chromosome 22 in both tumours is not pure coincidence but reflects a common mechanism of tumorigenesis.

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1. Bishop, J. M. *Rev. Biochem.* **52**, 301-354 (1983).
2. Koufos, A. *et al. Nature* **309**, 170-172 (1984).
3. Orkin, S. H., Goldman, D. S. & Sallan, S. E. *Nature* **309**, 172-174 (1984).
4. Reeve, A. E. *et al. Nature* **309**, 174-176 (1984).
5. Fearon, E. R., Vogelstein, B. & Feinberg, A. P. *Nature* **309**, 176-178 (1984).
6. Cavenee, W. K. *et al. Nature* **305**, 779-784 (1983).
7. Dryja, T. P. *et al. New Engl. J. Med.* **310**, 550-553 (1984).
8. Schoenberg, B. S., Christine, B. W. & Whisnant, J. P. *Am. J. Epidemiol.* **104**, 499-510 (1976).
9. Eldridge, R. *Adv. Neurol.* **29**, 57-65 (1981).
10. Martuza, R. L. & Ojemann, R. G. *Neurosurgery* **10**, 1-12 (1982).
11. Kanter, W. R., Eldridge, R., Fabricant, R., Allen, J. C. & Koerber, T. *Neurology* **30**, 851-859 (1980).
12. Huson, S. M. & Thrush, D. C. *Q. J. Med.* **218**, 213-224 (1985).
13. Zang, K. D. *Cancer Genet. Cytogenet.* **6**, 249-274 (1982).
14. Mark, J. *Adv. Cancer Res.* **124**, 165-222 (1977).
15. Zankl, H. & Zang, K. D. *Cancer Genet. Cytogenet.* **1**, 351-356 (1980).
16. Rubenstein, L. J. *Tumors of the Central Nervous System* (Armed Forces Institute of Pathology, Washington, DC, 1972).
17. Koufos, A. *et al. Nature* **316**, 330-334 (1985).
18. Hansen, M. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 6216-6220 (1985).
19. Davidoff, L. M. & Martin, J. J. *Neurosurg.* **12**, 375-384 (1955).

20. Gardner, W. J. & Turner, O. *Archs. Neurol. Psychiat.* **41**, 76-99 (1940).
21. Breakefield, X. O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4213-4216 (1984).
22. Darby, J. K. *et al. Am. J. hum. Genet.* **37**, 52-59 (1985).
23. Gusella, J. F. *et al. Nature* **306**, 234-238 (1983).
24. Gusella, J. F. *et al. Nature* **318**, 75-78 (1985).
25. Gusella, J. F. *J. clin. Invest.* (in the press).
26. Willard, H., Scolnick, M. H., Pearson, P. L. & Mandel, J. L. *Cytogenet. cell. Genet.* **40**, 360-489 (1985).
27. Barker, D. *et al. Molec. biol. Med.* **1**, 199-206 (1983).
28. Bell, G. I., Horita, S. & Karam, J. H. *Diabetes* **33**, 176-183 (1984).
29. Cavenee, W., Leach, R., Mohandas, T., Pearson, P. & White, R. *Am. J. hum. Genet.* **36**, 10-24 (1984).
30. Dryja, T., Rapaport, J. M., Weichselbaum, R. & Bruns, G. A. P. *Hum. Genet.* **65**, 320-324 (1984).
31. Wyman, A. R. & White, R. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6754-6758 (1980).
32. Chakravarti, A., Phillips III, J. A., Mellits, K. H., Buetow, K. H. & Seeburg, P. H. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6085-6089 (1984).
33. Davies, K. *et al. J. med. Genet.* **20**, 259-263 (1983).
34. Stewart, G. D., Harris, P., Galt, J. & Ferguson-Smith, M. A. *Nucleic Acids Res.* **13**, 4125-4132 (1985).
35. Barker, D. & White, R. *Cytogenet. cell. Genet.* **37**, 250 (1984).
36. Julier, C. *et al. Cytogenet. cell. Genet.* **40**, 664 (1985).
37. Barker, D., Schafer, M. & White, R. *Cell* **36**, 131-138 (1984).

Tissue-specific expression of the human tropomyosin gene involved in the generation of the *trk* oncogene

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The *trk* oncogene is a human transforming gene generated by the fusion of tropomyosin gene sequences to a truncated tyrosine kinase receptor gene¹. We have now characterized the normal tropomyosin gene from which the *trk* oncogene is derived. At least two different transcripts are expressed by this gene using a tissue-specific alternative messenger RNA splicing mechanism: a 2.5-kilobase (kb) mRNA encoding a 248-amino-acid tropomyosin in human fibroblasts and a 1.3-kb mRNA encoding a 285-amino-acid tropomyosin in human skeletal muscle. The rearrangement which generates the *trk* oncogene preserves most of the tropomyosin-coding sequences of the normal gene, including exons alternatively spliced in muscle and non-muscle tissue. We therefore expect the *trk* oncogene to show a tissue-specific pattern of transforming activity. Correct expression of the *trk* oncogene can occur only in non-muscle tissues. In muscle tissue the oncogene would almost certainly be inactive, as splicing according to the alternative muscle pattern aborts synthesis of the tyrosine kinase domain.

We have described an RNA-copy pseudogene *hTM_{nm}-1* which defines a 2.5-kb mRNA encoding a 248-amino-acid tropomyosin in human fibroblasts². The pseudogene *hTM_{nm}-1* is colinear with the 2.5-kb fibroblast mRNA and very closely related but not identical in sequence to it. Similarly, the tropomyosin-coding sequences of *trk* were found to be very closely related but not identical to *hTM_{nm}-1* (ref. 1). This suggests that the RNA-copy pseudogene *hTM_{nm}-1*, the 2.5-kb fibroblast mRNA and the tropomyosin-coding sequences of *trk* all originate from a common tropomyosin gene which we will call the *hTM_{nm}* structural gene. This is confirmed by DNA sequence analysis of complementary DNA clones of the 2.5-kb mRNA (not shown) which demonstrates that the tropomyosin-coding sequences of *trk* are identical to those of the 2.5-kb mRNA.

Tropomyosin genes have been shown to express structurally distinct protein isoforms by a tissue-specific alternative mRNA splicing mechanism³⁻⁶. We have therefore examined the expression of the *hTM_{nm}* structural gene in different tissues by hybridization of *hTM_{nm}-1* to blots of human fibroblast and muscle RNA (Fig. 1). The 2.5-kb mRNA characterized pre-

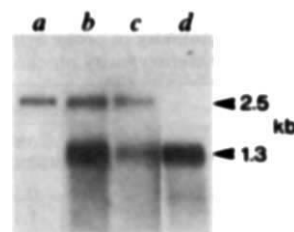
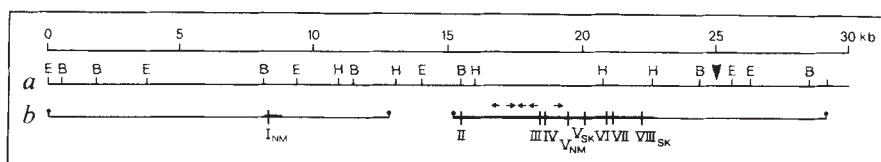


Fig. 1 Northern blot analysis of human non-muscle and muscle RNA. Poly (A)-containing RNA (0.5 µg) from cultured MRC-5 fibroblasts (lane a), adult heart muscle (lane b), fetal skeletal muscle (lane c) and adult skeletal (leg) muscle (lane d) was denatured with formaldehyde, subjected to electrophoresis and blotted onto nitrocellulose⁹. The filter was probed with M29, a probe derived² from the coding region of *hTM_{nm}-1*. Hybridization was carried out under stringent conditions (50% formamide, 42 °C) followed by washing in 0.2×SSC at 67 °C. The human fibroblast and adult skeletal muscle tracks were exposed to film for 24 h, the other tracks for 96 h. Sizes of the mRNAs were determined by comparison with known mRNA standards.

viously is found in the fibroblast, heart and fetal muscle RNA samples. In all the muscle preparations an additional 1.3-kb mRNA is apparent which cross-hybridizes with *hTM_{nm}-1* under stringent conditions. This transcript is particularly abundant in adult skeletal muscle, in which the 2.5-kb fibroblast mRNA is undetectable. Clones containing the complete coding sequence of the 1.3-kb muscle mRNA were isolated from a library of human skeletal muscle cDNA using *hTM_{nm}-1* as a probe (Fig. 2). The deduced protein sequence of this mRNA is 285 amino acids long (numbering from the initiator methionine) and is very closely related to the protein sequence of rabbit skeletal muscle α -tropomyosin⁷.

Comparison with the tropomyosin-coding sequences of *trk* reveals a very striking homology (Fig. 2). The two mRNAs encode distinct N-terminal protein sequences; that of the 1.3-kb mRNA is characteristic of a 284-amino-acid skeletal muscle tropomyosin while the N-terminal protein sequence of *trk* is characteristic of a 247-amino-acid cytoskeletal tropomyosin⁸. However, the nucleotide sequences encoding amino acids 80–188 and 214–257 of the 1.3-kb muscle mRNA are identical to sequences found in *trk* and the 2.5-kb fibroblast mRNA. These two regions of sequence identity are interrupted by a short region of non-identity encoding amino acids 189–213 of the muscle tropomyosin. This pattern of regions of nucleotide sequence identity interrupted by regions of nucleotide sequence non-identity is characteristic of transcripts derived from a common structural gene by alternative mRNA splicing⁶.

Fig. 2 Restriction map of a region of the *hTM_{nm}* structural gene. Probes derived from the pseudogene *hTM_{nm}-1* were used to screen libraries of human fibroblast DNA. These libraries were constructed as described elsewhere² using partial digestion with *Sau*3A, *Hin*dIII or *Bgl*II to generate the 15–20-kb DNA



fragments suitable for insertion into λ 1059 (ref. 12) or λ 2001 (ref. 13). Both clones were isolated from the partial *Bgl*II library. *a*, Restriction enzyme mapping was carried out with *Bam*HI (B), *Hin*dIII (H) and *Eco*RI (R). *b*, Positions of exons containing tropomyosin-encoding sequences. Although the clones do not overlap, a single *Bam*HI fragment spanning the 2.5-kb gap was found in Southern blots of *Bam*HI-digested human fibroblast DNA using single-copy probes from the isolates. The large arrow indicates the approximate point of divergence of the *trk* map from the normal *hTM_{nm}* gene; *trk* contains the *Bam*HI site on the left of the arrow but does not contain the *Eco*RI sites on the right side of the arrow¹. The small arrows indicate the position and orientations of the *Alu* repeat sequences located by DNA sequencing. The thick lines indicate the regions sequenced, and the vertical bars indicate the positions of the exons within this sequence. Exons containing the *trk* and 2.5-kb fibroblast mRNA sequences were located by comparison of the DNA sequence with that of *hTM_{nm}-1*. Exons containing other tropomyosin-coding sequences were identified by comparing three phase protein translations of the DNA sequence with all known tropomyosin sequences¹⁴ using the program DIAGON¹⁵. The *trk* sequence varies from the normal gene sequence at two positions (337 and 456), one of which (337) leads to a Lys $\rightarrow$ Glu amino-acid replacement. Where it is possible to make the comparison, the 1.3-kb muscle mRNA sequence is identical, base for base, with the normal gene sequence.

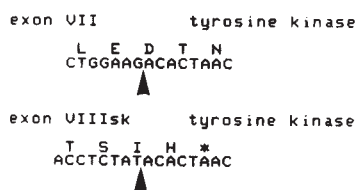


Fig. 4 Splicing according to a non-muscle pattern (top) with fusion of exon VII to the tyrosine kinase produces the correct frame of protein translation. Splicing according to a muscle pattern (bottom) with fusion of exon VIII_{sk} to the tyrosine kinase changes the frame of translation and results in the appearance of an in-frame stop codon only five nucleotides downstream from the splice junction (arrowhead).

are those found in the 2.5-kb fibroblast mRNA except that exon VII is joined to tyrosine kinase sequences to generate the transforming protein (Fig. 4).

How would *trk* behave if it were expressed in muscle tissue using the exons found in the 1.3-kb muscle mRNA? Certainly the tropomyosin sequence expressed by *trk* would be fundamentally altered. In particular, we would expect exon VII to be joined to exon VIII_{sk}, as it would be in the normal muscle tropomyosin, rather than directly to the tyrosine kinase sequence. Subsequent joining of exon VIII_{sk} to the tyrosine kinase sequence could occur but this would change the reading

frame of the *trk* sequence and abort the synthesis of the tyrosine kinase domain (Fig. 4). Therefore, we expect that *trk* will show a tissue-specific pattern of transforming activity, being active only in tissues which also express the non-muscle tropomyosin. This arises as a direct consequence of the alternative mRNA splicing mechanism which governs expression of the normal *hTM_{nm}* structural gene.

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1. Martin-Zanca, D., Hughes, S. H. & Barbacid, M. *Nature* **319**, 743-748 (1986).
2. MacLeod, A. R. & Talbot, K. *J. molec. Biol.* **167**, 523-537 (1983).
3. Karlik, C. C., Mahaffey, J. W., Couto, M. D. & Fyrberg, E. A. *Cell* **37**, 469-481 (1984).
4. Basi, G. S., Boardman, M. & Storti, R. V. *Molec. cell. Biol.* **4**, 2828-2836 (1984).
5. Ruiz-Opazo, N., Weinberger, J. & Nadal-Ginard, B. *Nature* **315**, 67-70 (1985).
6. MacLeod, A. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 7835-7839 (1985).
7. Stone, D. & Smillie, L. B. *J. biol. Chem.* **253**, 1137-1148 (1978).
8. Lewis, W. G., Coté, G. P., Mak, A. S. & Smillie, L. B. *FEBS Lett.* **156**, 269-273 (1983).
9. Scheller, R. H. *et al. Cell* **28**, 707-719 (1982).
10. MacLeod, A. R. *Nucleic Acids Res.* **9**, 2675-2689 (1981).
11. Henikoff, S. *Gene* **28**, 351-359 (1984).
12. Karn, J., Brenner, S., Barnett, L. & Cesarini, G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 5172-5176 (1980).
13. Karn, J., Matthes, H. W. D., Gait, M. J. & Brenner, S. *Gene* **32**, 217-234 (1984).
14. Sanders, C. & Smillie, L. B. *J. biol. Chem.* **260**, 7264-7275 (1985).
15. Staden, R. *Nucl. Acids. Res.* **10**, 2951-2961 (1982).

Sequences similar to the I transposable element involved in I-R hybrid dysgenesis in *D. melanogaster* occur in other *Drosophila* species

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The I factor is a transposable element controlling the I-R system of hybrid dysgenesis in *Drosophila melanogaster*. This phenomenon occurs when males from the inducer class of strains are crossed with females from the reactive class of strains¹. Inducer strains contain complete 5.4-kilobase (kb) I factors, reactive strains do not^{2,3}. Incomplete I elements are present in peri-centromeric regions of both categories of strains². The 5.4-kb I factors are genetically active in stimulating hybrid dysgenesis and can transpose, whereas incomplete I elements seem to be genetically inactive and transpose rarely. The results of *in situ* hybridization and Southern transfer experiments indicate that most incomplete I elements are at constant locations in all *D. melanogaster* populations, suggesting that they were present in the genome before the spread of this species throughout the world. To investigate the evolutionary origin of the I factor, we have studied various *Drosophila* species for the presence of sequences homologous to it. We find that such sequences are widespread. Moreover, elements that are very similar to complete and active I factors occur in the species most closely related to *D. melanogaster*.

Species of the genus *Drosophila* have been assigned to several subgenera, the largest of which are the *Sophophora* and the *Drosophila*. *D. melanogaster* is a species of the subgenus *Sophophora*, which is divided into four groups, *obscura*, *saltans*, *willistoni* and *melanogaster*, the last one being divided in various subgroups⁴. We have studied some species of the subgenera *Sophophora*, *Drosophila* and *Dorsilopha* for the presence of I homologues. The results (Table 1) show that such sequences are not restricted to *D. melanogaster*. We have found that, except

for *D. takahashii*, the DNAs of all the species studied in the *melanogaster* group show homology to the I factor. Sequences homologous with I seem to be much less frequent outside this group, for homology has been detected in only one species among eight tested in the other groups of the subgenus *Sophophora* and one among five in the other two subgenera. Sequences homologous to the I factor also occur in much more distant species such as *Scaptomyza pallida*, which belongs to another genus.

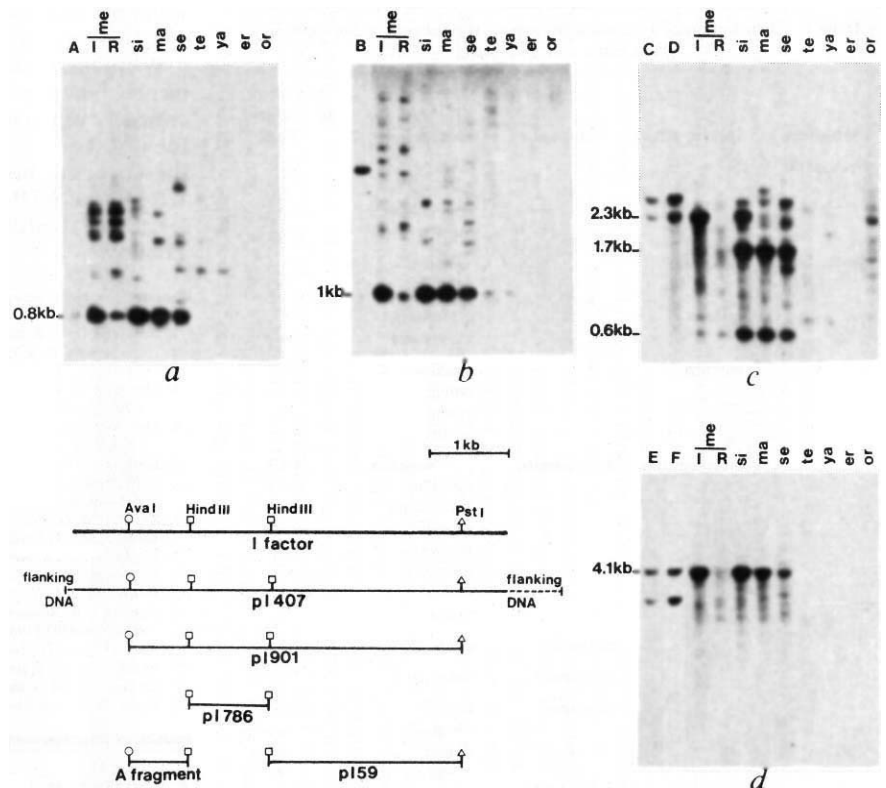
Within the *melanogaster* subgroup, the homology with the I factor is strongest in *D. simulans*, *D. mauritiana* and *D. sechellia*, weaker in *D. teissieri* and *D. yakuba*, and weaker still in *D. erecta* and *D. orena*. This correlates very well with the phylogenetic relationships between these species⁵.

Experiments were carried out to investigate the structure of the elements present in these species and to try to determine whether they are similar to the complete I factors of the inducer strains of *D. melanogaster*. Bucheton *et al.*² have shown that restriction fragments from within the I factor are more abundant in DNA from inducer strains than from reactive strains. This is particularly true for the 2.3-kb *HindIII*-*PstI* internal fragment of I (see Fig. 1). Therefore, we used clone p159, which contains this fragment, to probe *HindIII*-*PstI* digests of genomic DNA from stocks of various species of the *melanogaster* subgroup.

Figure 1c shows that, as reported previously, the inducer strains of *D. melanogaster* contain several copies of the 2.3-kb fragment per haploid genome whereas the reactive strains do not contain it. This provides a simple means of distinguishing between reactive and inducer strains in Southern blot experiments. There is no 2.3-kb fragment hybridizing to this probe in DNA from *D. erecta*, *D. orena*, *D. yakuba* and *D. teissieri*, but the DNA from *D. simulans* gives a similar pattern to that obtained with the inducer strains of *D. melanogaster*. Indeed, this species gives a 2.3-kb fragment which hybridizes strongly to the probe as well as two smaller fragments of 1.7 and 0.6 kb. Experiments which are not reported here indicate that these smaller fragments result from the presence, in some elements, of an additional *HindIII* restriction site within the *HindIII*-*PstI* fragment. Very similar results are obtained with *D. mauritiana* and *D. sechellia* and in both cases many I elements have this additional *HindIII* restriction site.

Fig. 1 Hybridization of various digests c DNAs from the species of the *melanogaster* subgroup with different internal fragments of the I factor. *a*, *Ava*I-*Hind*III digests probed with I factor fragment A; *b*, *Hind*III digests probed with p1786; *c*, *Hind*III-*Pst*I digests probed with p159; *d*, *Ava*I-*Pst*I digests probed with p1901. The strong signal observed for *D. orena* in *c* is due to overloading of DNA in the gel. For *D. erecta* and *D. orena*, signals can be seen with longer exposures. The names of the species are given above each lane with the following abbreviations: I, inducer strain Canton-S; R, reactive strain Charolles; me, *D. melanogaster*; si, *D. simulans*; ma, *D. mauritiana*; se, *D. sechellia*; te, *D. teissieri*; ya, *D. yakuba*; er, *D. erecta*; or, *D. orena*.

Methods. DNAs were digested with various restriction enzymes, electrophoresed on 1% agarose gels, transferred to nitrocellulose filters and hybridized with probes corresponding to various parts of the I factor (see Table 1 legend for hybridization and washing conditions). A restriction map of the I factor, and the clones bearing different parts of it which were used as probes, is given under the autoradiographs. Flanking DNA in p1407 is from the *white* gene. In track A an *Ava*I-*Hind*III digest of p1901 was loaded in amounts equivalent to about one copy of the 0.8-kb *Ava*I-*Hind*III fragment per haploid genome. Track B contains *Hind*III digests of p1786 in amounts equivalent approximately to one copy per haploid genome of the 1-kb *Hind*III fragment. In tracks C and D a *Hind*III-*Pst*I digest of clone p159 was loaded in amounts equivalent, respectively, to about one and five copies of the 2.3-kb *Hind*III-*Pst*I fragment. Tracks E and F contain *Ava*I-*Pst*I digests of p1901 in amounts equivalent respectively, to one and five copies per haploid genome of the 4.1-kb *Ava*I-*Pst*I fragment.



In other experiments, the same genomic DNAs were digested with *Ava*I and *Hind*III, and probed with the 0.8-kb fragment A of the I factor which had been gel purified from clone p1901 (Fig. 1a), or with *Hind*III and probed with clone p1786 which contains the 1-kb *Hind*III internal fragment of the I factor (Fig. 1b). Both of these internal fragments are present in several copies in reactive and inducer strains of *D. melanogaster* as well as in *D. simulans*, *D. mauritiana* and *D. sechellia*, but not in the other species.

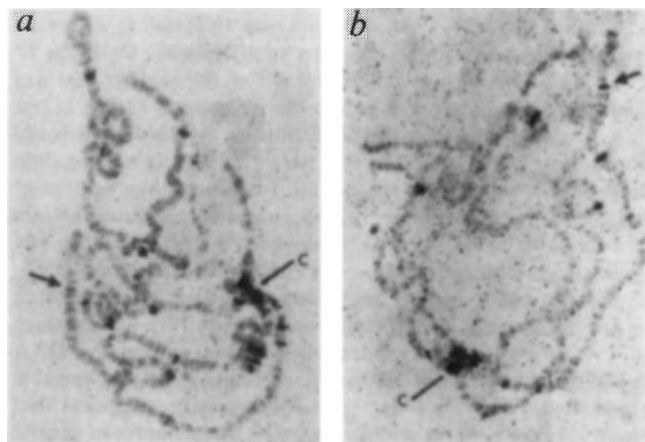


Fig. 2 Location of I factor sequences on *D. simulans* (a) and *D. mauritiana* (b). ^3H -labelled p1407 was hybridized to chromosome squashes as described previously². In each case C shows hybridization to the chromocentre. The arrow indicates region 3C, which contains the *white* locus. Hybridization in this region is thought to be caused by the *white* sequences carried by clone p1407 (see Fig. 1).

In the last Southern blot experiment, the DNAs were digested with *Ava*I and *Pst*I and hybridized with clone p1901. This digestion produces a large 4.1-kb internal fragment (see Fig. 1). The results shown in Fig. 1d suggest that this large fragment is present in several copies only in the inducer strains of *D. melanogaster* and in the sibling species *D. simulans*, *D. mauritiana* and *D. sechellia*, indicating that several I homologues in these species contain most of the sequence of the complete I factor.

We have studied the distribution of I elements in the genomes of *D. simulans* and *D. mauritiana* by *in situ* hybridization to salivary gland chromosomes of the larvae. The results (Fig. 2) indicate that, as for the inducer strains of *D. melanogaster*, these sequences are present in both the peri-centromeric regions and chromosome arms of these species.

All the above results indicate that the stocks of *D. simulans*, *D. mauritiana* and *D. sechellia* used in these experiments differ from reactive strains of *D. melanogaster* and look more like the inducer strains. The sequences found in these species which are homologous to the I factor are strikingly similar to the functional I factors found in the inducer strains.

The survey of various *Drosophila* species reported in Table 1 indicates that I elements are an old component of the genomes and were probably present before the radiation of the *melanogaster* group. Many results suggest that in *D. melanogaster* the complete I factor appeared in natural populations in the 1930s or 1940s^{6,7}. One hypothesis advanced to explain the relationships between incomplete I elements and complete I factors is that incomplete I elements could be the result of mutational inactivation of an old transposable element, and that the complete I factor could have been formed by reactivation of some of these incomplete and defective I elements, perhaps by recombination². Such a reactivation could have occurred in one of the four species bearing potentially active I factors—*D. melanogaster*, *D. simulans*, *D. mauritiana* or *D. sechellia*—but

Table 1 Distribution of I homologues among the subgenera *Sophophora*, *Drosophila* and *Dorsilopha*

Subgenus	Species group	Subgroup	Species	Homology to I factor DNA
<i>Drosophila</i>			<i>virilis</i>	—
			<i>funnebris</i>	—
			<i>immigrans</i>	—
			<i>fraburu</i>	—
<i>Sophophora</i>	<i>willistoni</i>		<i>willistoni</i>	—
			<i>nebulosa</i>	—
	<i>saltans</i>		<i>prosaltans</i>	—
			<i>sturtevantii</i>	+
	<i>obscura</i>		<i>obscura</i>	—
			<i>ambigua</i>	—
			<i>guanche</i>	—
			<i>bifasciata</i>	—
	<i>melanogaster</i>	<i>melanogaster</i>	<i>melanogaster</i>	+++
			<i>simulans</i>	+++
			<i>mauritiana</i>	+++
			<i>sechellia</i>	+++
			<i>teissieri</i>	++
			<i>yakuba</i>	++
			<i>erecta</i>	+
			<i>orena</i>	+
			<i>eugracilis</i>	+
			<i>takahashii</i>	—
			<i>montium</i>	++
			<i>burlai</i>	+
			<i>bocqueti</i>	+
			<i>davidi</i>	+
			<i>serrata</i>	+
			<i>malagassya</i>	++
			<i>vulcana</i>	+
			<i>kikkawai</i>	+
			<i>tsakasi</i>	+
			<i>bakoue</i>	+
	<i>ananassae</i>		<i>ananassae</i>	++
			<i>malerkotliana</i>	+
<i>Dorsilopha</i>			<i>busckii</i>	+

DNA from various *Drosophila* species was prepared from adult flies and digested with restriction enzymes. The fragments were electrophoresed on agarose gels, transferred to nitrocellulose filters¹⁴ and hybridized with clones bearing various parts of the I factor, usually pI901 and pI407 (see Fig. 1), and labelled with ³²P by nick translation¹⁵ to a specific activity of 3–10 × 10⁷ d.p.m. per µg. Hybridization conditions were 1 × Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 4 × SSC, 50% formamide for at least 16 h at 37 °C. The filters were washed for 2 h in 2 × SSC, 0.1% SDS at room temperature and then for a further 2 h in 1 × SSC, 0.1% SDS at 37 °C. The number of plus signs indicates the intensity of the signal observed on the autoradiographs. Most of the stocks were kindly provided by the Laboratoire de Génétique Evolutive of the CNRS at Gif-sur-Yvette (France).

It is unlikely that this occurred independently four times in each of the four species. It is more likely that the functional I factor formed only once in one species, and was then transmitted to the other three species. This could happen by horizontal transfer via an unknown vector. Another possibility resides in the fact that certain of these species can mate. The hybrids are usually sterile, but one can imagine that in such crosses some DNA sequence could be transmitted from one species to another as the result of very rare events such as polyspermy. However, the present data suggest strongly that the complete I factor was present in the ancestral species of *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia* and has been lost in *D. melanogaster* when this species diverged from the other three, possibly by drift. Then it could have re-invaded the *D. melanogaster* genome in the 1930s either by reactivation of defective I elements or by horizontal transfer from *D. simulans*.

The interspecific distribution of I elements is quite different from that of sequences homologous to the P factor, which is the transposable element involved in the P-M system of hybrid dysgenesis^{8,9}. Indeed, P homologues do not occur in the close relatives of *D. melanogaster*¹⁰ but are common in the *obscura*,

willistoni and *saltans* groups^{11–13}. This suggests that the two systems of hybrid dysgenesis have evolved in very different ways.

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1. Bregliano, J. C. & Kidwell, M. G. in *Mobile Genetic Elements* (ed. Shapiro, J.) 363–410 (Academic, New York, 1983).
2. Bucheton, A., Paro, R., Sang, H. M., Pélisson, A. & Finnegan, D. J. *Cell* **38**, 153–163 (1984).
3. Finnegan, D. J. & Fawcett, D. H. in *Oxford Surveys on Eucaryotic Genes* Vol. 3 (Oxford University Press, in the press).
4. Throckmorton, L. H. in *Handbook of Genetics* Vol. 3 (ed. King, R. C.) 421–469 (Plenum, New York, 1975).
5. Lemeunier, F., David, J., Tsacas, L. & Ashburner, M. in *The Genetics and Biology of Drosophila* Vol. 3e (eds Ashburner, M., Carson, H. L. & Thompson, J. N.) (Academic, London, in the press).
6. Kidwell, M. G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1655–1659 (1983).
7. Kidwell, M. G., Frydryk, T. & Novy, J. B. *Drosoph. Inf. Serv.* **59**, 63–69 (1983).
8. Engels, W. R., *A. Rev. Genet.* **17**, 315–344 (1983).
9. O'Hare, K. *Trends Genet.* **1**, 250–254 (1985).
10. Brookfield, J. F. Y., Montgomery, E. & Langley, C. H. *Nature* **310**, 330–332 (1984).
11. Daniels, S. B., Strausbaugh, L. D., Ehrman, L. & Armstrong, R. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6794–6797 (1984).
12. Anxolabehere, D., Nouaud, D. & Périquet, G. *Genet. Select. Evol.* **17**, 579–584 (1985).
13. Lansman, R. A., Stacey, S. N., Grigliatti, T. A. & Brock, H. W. *Nature* **318**, 561–563 (1985).
14. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
15. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. J. *J. molec. Biol.* **113**, 237–251 (1977).

Cryptic simplicity in DNA is a major source of genetic variation

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DNA regions which are composed of a single or relatively few short sequence motifs usually in tandem ('pure simple sequences') have been reported in the genomes of diverse species (for reviews see refs 1–4), and have been implicated in a range of functions including gene regulation (for reviews see refs 5–7), signals for gene conversion and recombination^{8–10}, and the replication of telomeres¹¹. They are thought to accumulate by DNA slippage^{12–16} and mispairing during replication and recombination or extension of single-strand ends^{2,4,10,11}. In order to systematize the range of DNA simplicity and the genetic nature of the regions that are simple, we have undertaken an extensive computer search of the DNA sequence library of the European Molecular Biology Laboratory (EMBL)¹⁷. We show here that nearly all possible simple motifs occur 5–10 times more frequently than equivalent random motifs. Furthermore, a new computer algorithm reveals the widespread occurrence of significantly high levels of a new type of 'cryptic simplicity' in both coding and noncoding DNA. Cryptically simple regions are biased in nucleotide composition and consist of scrambled arrangements of repetitive motifs which differ within and between species. The universal existence of DNA simplicity from monotonous arrays of single motifs to variable permutations of relatively short-lived motifs suggests that ubiquitous slippage-like mechanisms are a major source of genetic variation in all regions of the genome, not predictable by the classical mutation process.

To assess the frequencies and types of simple sequence motifs, we made a systematic survey of published sequences¹⁷ using

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Table 1 Number of matches with simple sequence probes found in the EMBL library of DNA sequences (a) and in different groups of organisms (b)

a, Matches with mononucleotide motifs										
	AA/TT	GG/CC		AT/AT	Matches with simple dinucleotide motifs					
					GC/GC	GT/AC	GA/TC			
13/15	196	43	13/15	26	4	41	43			
14/15	74	8	14/15	6	—	22	26			
15/15	36	2	15/15	1	—	12	16			
Matches with random dinucleotide motifs										
			13/15	22.3	7.3	2.5	7.5			
			14/15	2.5	0.3	—	0.3			
			15/15	0.3	—	—	—			
Matches with simple trinucleotide motifs										
	AAT/ATT	AAC/GTT	AAG/CTT	ATG/CAT	TAG/CTA	CCT/AGG	CCA/TGG	CGA/TCG	GCA/TGC	CCG/CGG
	ATA/TAT	ACA/TGT	AGA/TCT	TGA/TCA	AGT/ACT	CTC/GAG	CAC/CTG	GAC/GTC	CAG/CTG	CGC/GCG
	TAA/TTA	CAA/TTG	GAA/TTT	GAT/ATC	GTA/TAC	TCC/GGA	ACC/GGT	ACG/CGT	AGC/GCT	GCC/GGC
13/15	80	30	49	25	12	59	43	10	64	49
14/15	17	5	13	7	1	26	5	2	17	11
15/15	8	2	3	2	—	4	1	1	4	4
Matches with random trinucleotide motifs										
13/15	19.0	7.8	11.5	1.0	1.0	9.0	4.5	2.5	1.0	11.0
14/15	1.5	0.3	0.8	—	—	0.5	0.3	—	—	1.3
15/15	—	—	—	—	—	—	—	—	—	—

b, No. of matches per 100 kb										
		Mononucleotide motifs			Dinucleotide motifs			Trinucleotide motifs		
Vertebrates	13/15			18.1			11.0			23.7
645 kb	14/15			6.5			6.7			7.3
	15/15			3.7			3.6			2.0
Non-vertebrates	13/15			26.7			7.5			29.2
240 kb	14/15			11.7			2.1			6.3
	15/15			4.2			0.8			2.9
Plants & algae	13/15			11.4			5.7			31.4
35 kb	14/15			8.5			5.7			5.7
	15/15			2.8			5.7			2.8
Chloroplasts	13/15			26.6			6.6			10.0
30 kb	14/15			3.3			—			—
	15/15			—			—			—
Mitochondria	13/15			10.0			7.0			30.0
100 kb	14/15			2.0			—			4.0
	15/15			—			—			—
Viruses	13/15			5.5			1.8			18.9
550 kb	14/15			0.9			0.7			5.3
	15/15			0.5			0.4			1.5
Prokaryotes	13/15			1.3			1.3			11.9
320 kb	14/15			0.3			—			1.9
	15/15			—			—			—
Phages	13/15			1.2			—			7.1
170 kb	14/15			—			—			0.6
	15/15			—			—			—
Matches with random probes										
Whole library	13/15						1.9			3.2
2,090 kb	14/15						0.1			0.2
	15/15						0.0			—

a. The library was searched using the MATCH program³⁵ on the Cambridge IBM 3081 computer. We used 15-nucleotide (nt)-long probe sequences with matches of 13 out of 15 nt (13/15), 14 out of 15 nt (14/15) and complete matches (15/15). The actual number of matches found with each of the simple sequence probes is shown. For matching sequences longer than 15 base pairs, only one match is recorded. All possible combinations of one, two and three nucleotides were tested. In the case of the trinucleotide repeats, only one of the three possible permutations (which are indicated in the headline) was tested. The results from the runs with complementary strands and the runs with RNA probes (U instead of T) were merged. Matches with yeast mitochondrial sequences were excluded from this search because of their unusual nucleotide composition (80–90% AT). Matches due to duplicate entries of the same sequences in the library were also excluded. Testing with randomized probes was done at least four times for each nucleotide combination (two for each strand) and the values given are averages. Standard deviations are in the order of 3 for the 13/15 matches and 1 for the 14/15 matches. **b.** The values represent the number of matches found per 100 kb in each organism group and are therefore directly comparable. For the calculation of the proportional representation of each group of organisms, yeast mitochondrial sequences were excluded, but duplicate entries could not be excluded. It is thought, however, that this leads to an insignificant error.

two criteria. The first was to scan for direct sequence homologies to 15-nucleotide-long probes, each consisting of an array of one of all possible mono-, di- and trinucleotide motifs. Three different stringencies of homology were recorded: 13 out of 15, 14 out of 15 and 15 out of 15 bases matched. In test runs using random sequence motifs of balanced nucleotide composition, we found an average of four matches with 13/15 in the whole library (~2,100 kilobases; kb) and none with either 14/15 or 15/15. Thus, a 13/15 match could be considered as being at the borderline of significance. Controls consisted of randomized probes with equivalent nucleotide compositions.

The results (Table 1; see legend for details of the tests) indicate that nearly all simple sequence motifs are considerably more

frequent than their randomized counterparts (Table 1a). The only notable exceptions are the AT/AT and GC/GC motifs. With respect to the latter, there is a known suppression of CG dinucleotides in eukaryotic nuclear DNA¹⁸. However, the level of suppression probably cannot account for the insignificant representation of this motif in simple dinucleotide repeats. Furthermore, the trinucleotide repeats composed of C and G (column CCG/CGG in Table 1a) are fairly frequent and fit into the overall frequency pattern of other trinucleotide repeats. Although it is possible that tandem arrays of GC/GC motifs are suppressed because of their potential to form Z-DNA⁵, GT/AC repeats can form the same structures and seem not to be particularly suppressed. In general, it does not seem as if

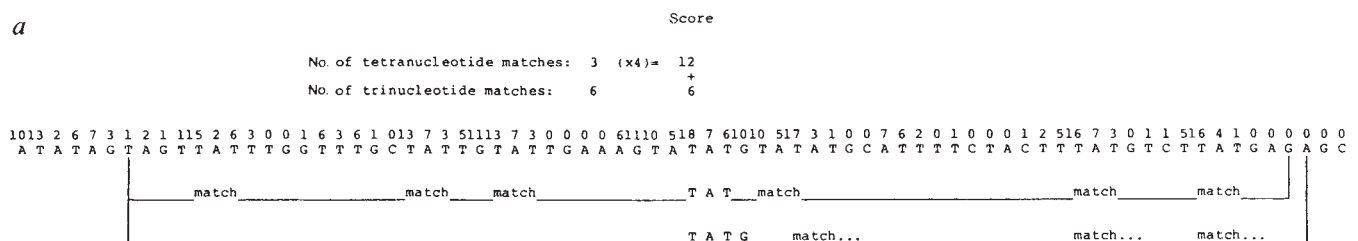
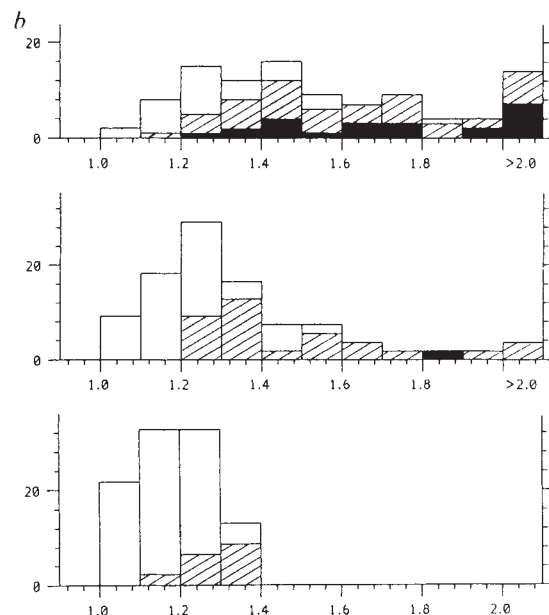


Fig. 1 a. The computer algorithm. The program counts how often the oligonucleotide motif which starts to the right of each nucleotide is repeated in its direct neighbourhood. A range of parameters (motif length and window length) were tested on randomized sequences. Our actual motif and window lengths were chosen as a balance between reducing background noise and optimizing the match signal. We chose tri- and tetranucleotide motifs and a window of ± 32 nucleotides. In a random sequence consisting of 25% of each nucleotide, one would expect 1 match with a trinucleotide and 1/4 match with a tetranucleotide within 64 nt. Therefore, the number of tetranucleotide matches detected are multiplied by four in the program. The final score for the respective nucleotide is then the sum from both match comparisons. This is demonstrated for the T at position 39 in the figure. The rightward trinucleotide motif from this T is TAT and the tetranucleotide motif is TATG. It is indicated which region around this T is searched and where the matches were found. For six trinucleotide and three tetranucleotide matches a final score of 18 is calculated and assigned to the T at position 39. The program would then go onto the next nucleotide and repeat the cycle. Hence, each nucleotide is assigned a simplicity score. The figure also demonstrates the inbuilt correction system which eliminates double scorings due to overlapping matches. For the motif TATAT at position 43, only one match with TAT is recorded. Furthermore, the program obviates edge effects by not assigning scores to the first 32 nucleotides and the last 35 nucleotides of a sequence. The overall simplicity factor for a given sequence is calculated by summing all scores and dividing the sum by the number of nucleotides in the sequence. The relative simplicity factor is then obtained by dividing this overall simplicity factor by the overall simplicity factor obtained from 10 or 100 randomized sequences with the same nucleotide composition and a length of 10,000 nt each. Thus, the relative simplicity factor would be expected to be close to 1.0 for a sequence with no simplicity and higher for sequences with simple regions. Using 10 different runs of randomized sequences, we can calculate a standard deviation for the overall simplicity factors of the runs and use it to assess the significance of the simplicity factor of the natural sequence. Typical results from this comparison give a significance ($P < 0.01$) for relative simplicity factors larger than 1.1 to 1.2. The program was written in Fortran 77 and run on the Cambridge IBM 3081 computer. **b.** Comparison of the frequency of simplicity factor classes between eukaryotes and prokaryotes. The histograms show the percentage of each class of simplicity factors found in sequences from eukaryotes (upper), eukaryotic cDNAs (middle) and prokaryotes (lower). All tested sequences were larger than 1 kb and were taken randomly from the EMBL library of DNA sequences (release 5)¹⁷. For eukaryotes, 98 different sequences were tested, 52 for eukaryotic cDNAs and 46 for prokaryotic sequences. The hatched parts of the bars represent the fraction of those sequences which contain at least one region of high simplicity. The criterion for this was that in the graphical display of the simplicity profile, at least one peak had to be higher than the line which indicates the highest peak from any of the randomized sequences of equivalent composition (the broken reference line, in Fig. 2). The filled parts of the bars represent the fraction of those sequences which contained a pure simple sequence. The criterion for this was that the sequence had to contain an uninterrupted pure simple sequence at least 15 nt long. Stretches of this length (or longer) would be expected to be detectable by direct hybridization experiments.



particular types of sequences (for example, polypurinic sequences¹⁹) are over- or under-represented.

Table 1b shows the average number of matches per 100 kb within the different species groups. The results clearly indicate that simple sequence matches are most frequent in eukaryotic nuclear genomes and very rare in prokaryotes. Chloroplasts, mitochondria and eukaryotic viruses occupy a somewhat intermediate position in this table.

In order to detect accumulations of direct oligonucleotide repeats which need not have a regular spacing (cryptic simplicity), we devised a computer program to search for such regions: Fig. 1a outlines how it works. A sequence is given an overall 'simplicity factor', which is a measure of the combined frequencies of naturally occurring, directly repeated motifs in the sequence (see Fig. 1 legend for details of the algorithm). The profile of simplicity of a given region can be displayed graphically. In addition, the program calculates a 'relative simplicity factor', which is the ratio of the simplicity factor of a test sequence to the mean simplicity factor calculated from 10 or 100 randomized sequences each consisting of 10 kb of equivalent nucleotide composition. Standard deviations were found to be very small under these conditions. The relative simplicity factor is expected to be close to 1.0 for a sequence with no cryptic simplicity, while relative simplicity factors above

1.1–1.2 are significant ($P < 0.01$) under our test conditions (see Fig. 1 legend).

We have tested a few hundred natural sequences with the program and found that most of them have a relative simplicity factor which is clearly higher than 1.0. As with the previous method of analysis, there is a clear difference between eukaryotic and prokaryotic sequences. The simplicity factors of the latter are clustered within a range of 1.0–1.4 (Fig. 1b, bottom), and the eukaryotic sequences are spread more evenly within a range of up to 2.0 and occasionally higher (Fig. 1b, top). Complementary DNA sequences show a somewhat intermediate position (Fig. 1b, middle), as do viral sequences (not shown). In the case of the cDNA sequences, the high simplicity regions are not only confined to the 5' or 3' untranslated regions, but can be found within the coding regions themselves (see also Fig. 2). Most of the eukaryotic sequences tested have high simplicity factors even if they do not have long arrays of pure simple sequence motifs (Fig. 1b). This implies that cryptic simplicity (the frequent occurrence of several directly repeated short motifs in close proximity) is widespread and an important component of eukaryotic genomes.

Sophisticated comparative statistics have recently been described for measuring the significance of the occurrence of repetitive motifs and other non-random properties of DNA

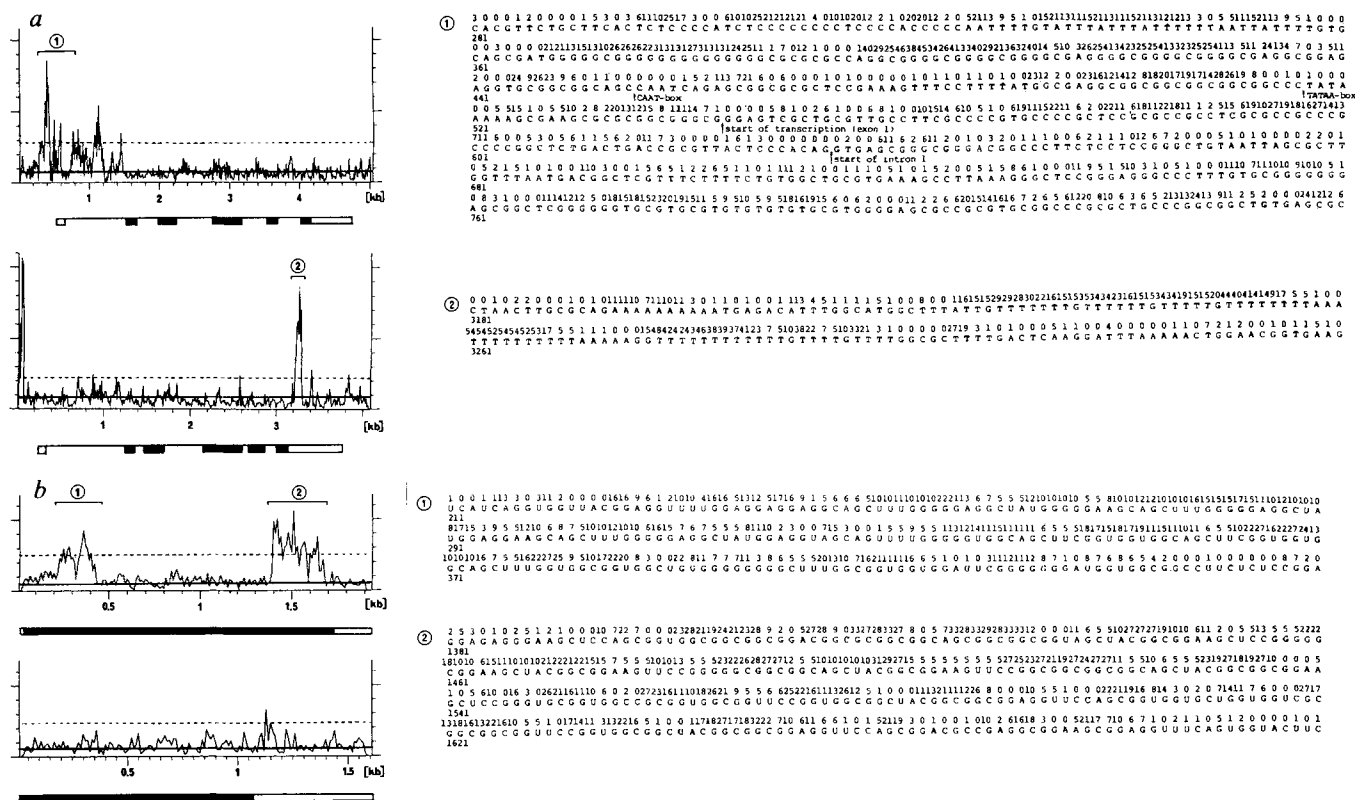


Fig. 2 Examples of high simplicity regions in eukaryotic genes. The figure shows the distribution of simple sequence regions across the genes. For this purpose, the scores from the simplicity factor calculations (averaged in 10-nt steps) are displayed in a graph above the appropriate positions in the sequence. The structure of the genes is displayed beneath the graphs. The thin line shows the length of the transcription unit, the open boxes are nontranslated parts of the mRNA and the filled boxes are the coding regions. The continuous horizontal line in the graphs is the level of the mean overall simplicity factor of 10 randomized sequences of similar composition to the test sequence. The broken line in the graphs shows the level of the highest peak in any one of the randomized sequences. Only peaks above this line are considered as high simplicity regions. Some of these were numbered and the corresponding sequences are shown to the right of the graph together with the simplicity scores found for each nucleotide. *a*, Upper part, chicken cytoplasmic actin gene (ref. 36, accession number in the EMBL library: X00182); lower part, rat cytoplasmic actin gene (ref. 37, accession no. V01217). *b*, Upper part, mouse keratin gene (cDNA) (ref. 24, accession no. V00830); lower part, human keratin gene (cDNA), the 5' end of the gene is not sequenced (ref. 38, accession no. V01516).

organization in defined sequences^{20,21}. It is satisfying that both these and our methods reveal that many (probably most) coding and noncoding sequences show significantly high numbers of short repetitive motifs, in a seemingly limitless number of permutations.

To determine whether new motifs are continually being generated on an evolutionary timescale, equivalent DNA regions were compared between species; Fig. 2 shows some characteristic examples. In the chicken cytoplasmic actin gene (Fig. 2a), a large region of simplicity composed of various different motifs stretches from a point upstream of the start of transcription and into most of the first intron. The same region in the rat gene has a far lower simplicity, even though it is also G+C-rich. Instead, there is a new high simplicity region in the 3' noncoding part of the messenger RNA which is based on oligo(T) stretches interspersed with Gs. It is unlikely that this arose by a recent integration of a retrotransposon²², because the sequences flanking it are partly conserved between chicken, rat and human and might actually be of functional significance²³. The large peak in front of the rat gene is also due to an oligo(T) stretch.

Figure 2b shows cryptic simple sequences in the coding sequences of the rat keratin gene. There are two glycine-rich regions which are highly divergent between the keratin genes, while the body of the gene, which our analysis indicates is not simple, is fairly strongly conserved, even between different types of intermediate filaments²⁴. The human keratin gene is much less simple in the equivalent regions (only part of the human gene sequence is known).

It has been noted previously that some regions of mammalian 28S ribosomal RNA genes are highly divergent in close species comparisons²⁵. Detailed examination of the distribution of cryptic simplicity in mouse and rat 28S rDNA shows that they coincide exactly and in very great detail with the regions of divergence in the two genes as depicted by standard dot matrices (Fig. 3). The regions in question are known to be insertions (when compared with other eukaryotic rRNA genes), probably originating by slippage-like mechanisms and not by the drift of point mutations in neutral sequences that were once shared by all species.

Our results show that there is a significantly frequent occurrence of nearly all possible short simple sequence motifs in natural DNA sequences in both coding and noncoding regions of eukaryotic genomes. There must be a mechanism active throughout most of the genome which can create simplicity, and which acts largely independently of the actual sequences involved. It is reasonable to assume that this mechanism is slippage. It is known that short oligonucleotide motifs can slip easily against each other⁷, for this is a very efficient way to produce simple sequence DNA *in vitro*^{14,15}. From direct comparisons of closely related natural DNA sequences, it is clear that slippage can also occur *in vivo* between short direct repeats, leading to deletions or expansions of the motifs involved (for examples see refs 4, 10, 12, 13, 16, 26–28).

The apparent under-representation of simplicity in prokaryotes is probably not due to the absence of such mechanisms, but merely to the more economical use of DNA in these

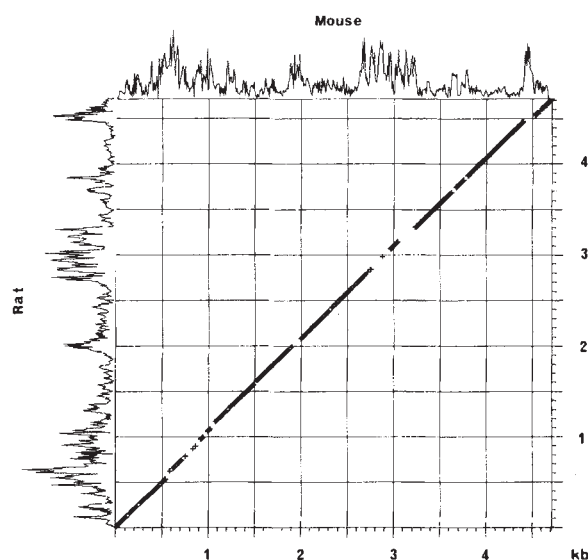


Fig. 3 Comparison of the genes for the 28S ribosomal RNA from mouse to rat. The figure shows a standard dot matrix plot between mouse³⁹ and rat⁴⁰ 28S rRNA genes. Match stringency was 20 nt perfect match. The plot is superimposed with the simplicity profiles of these genes, with the mouse gene on the upper axis and the rat on the left axis.

organisms, for they have low amounts of noncoding spacer DNA. Interestingly, prokaryotes also have a more frequent occurrence of simple trinucleotide motifs (compare Table 1), possibly due to their tolerance by the triplet code.

Two broad categories of simplicity can be distinguished—pure simple sequence regions and regions of less regularity but with a clear bias in nucleotide composition (compare Fig. 2). Ohno has suggested that the latter regions, where they occur within genes, might be the remnants of the former, which gradually diverged with the accumulation of point mutations²⁹. However, it is inconceivable that random point mutations within a simple sequence could by itself lead to the observed biases in nucleotide composition and to scrambling of new and old motifs. The observations can be more easily explained in terms of an interaction between point mutations and continual slippage.

The turnover of simple motifs could make some contribution to the observed differences in codon usage between genes and species, and in the potential of chromatin to undergo conformational changes and bind to regulatory proteins associated with gene transcription. Furthermore, it seems unlikely that substantial amounts of complex 'single-copy' DNA exist which are diverging in a clock-like manner, given that most DNA is subject to one or other turnover mechanism^{30,41}.

Mechanisms of continuous gain and loss of DNA variants by unequal crossing over and gene conversion can lead to the homogenization (molecular drive) of any given variant in a sexual population^{30–33,41}. No large-scale homogenization is occurring by gain and loss due to slippage because our survey shows that the unit of slippage can occasionally be out of phase with pre-existing motifs. Thus, any homogenization is continually being obliterated by the generation and combinatorial reshuffling of short and short-lived motifs differing in sequence and length. The effects on the overall genetic composition of a population and any interaction with selection are probably complex^{4,30,34,41}. It appears that the genetic material acquires its new forms more as a consequence of novel combinations between simple units than by the point-by-point substitutions of one base by another in a passive DNA molecule.

2. Tautz, D. & Renz, M. *Nucleic Acids Res.* **12**, 4127–4138 (1984).
3. Greaves, D. R. & Patient, R. K. *EMBO J.* **4**, 2617–2626 (1985).
4. Dover, G. A. & Tautz, D. *Phil. Trans. R. Soc.* **312**, 275–290 (1986).
5. Wang, A. J. H. *et al. Nature* **282**, 680–686 (1979).
6. Weintraub, H. & Groudine, M. *Science* **193**, 348–356 (1976).
7. Hentschel, C. C. *Nature* **295**, 714–716 (1982).
8. Shen, S. H., Slighton, J. L. & Smithies, O. *Cell* **26**, 191–203 (1981).
9. Goodman, M. *Bioessays* **3**, 9–14 (1985).
10. Jeffreys, A. J., Wilson, V. & Thein, S. L. *Nature* **314**, 67–72 (1985).
11. Blackburn, E. & Szostak, J. A. *Rev. Biochem.* **53**, 163–152 (1984).
12. Drake, J. W., Glickman, B. W. & Ripley, L. S. *Am. Sci.* **71**, 621–630 (1983).
13. Streisinger, G. *et al. Cold Spring Harb. Symp. quant. Biol.* **31**, 77–84 (1966).
14. Wells, R. D., Ohtsuka, E. & Khorana, H. G. *J. molec. Biol.* **14**, 221–240 (1965).
15. Morgan, A. R. *et al. Biochemistry* **13**, 1596–1603 (1974).
16. Efstratiadis, A. *et al. Cell* **21**, 653–668 (1980).
17. Cameron, G. *et al. EMBL Sequence Data Library Release 4* (1985).
18. Bird, A. P. *Nucleic Acids Res.* **8**, 1499–1504 (1980).
19. Straus, N. A. & Birnboim, H. C. *Biochim. biophys. Acta* **454**, 419–428 (1976).
20. Karlin, S. & Ghandour, G. *Proc. natn. Acad. Sci. U.S.A.* **5800**–5804 (1985).
21. Karlin, S., Ghandour, G. & Foulser, D. E. *Molec. Biol. Evol.* **2**, 35–45 (1985).
22. Rogers, J. *Nature* **305**, 101–102 (1983).
23. Yaffe, D. *et al. Nucleic Acids Res.* **13**, 3723–3737 (1985).
24. Steinert, P. M. *et al. Nature* **302**, 794–800 (1983).
25. Hassouna, N., Michot, B. & Bachellerie, J. P. *Nucleic Acids Res.* **12**, 3563–3583 (1984).
26. Moore, G. P. *Trends biochem. Sci.* **8**, 411–414 (1983).
27. Jones, W. C. & Kafatos, F. C. *J. molec. Evol.* **19**, 87–103 (1982).
28. Brown, S. D. M. & Piechaczyk, M. *J. molec. Biol.* **165**, 249–256 (1983).
29. Ohno, S. & Epplen, J. T. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3391–3395 (1983).
30. Dover, G. A. *Nature* **299**, 1121–119 (1982).
31. Coen, E. S., Thoday, J. M. & Dover, G. A. *Nature* **295**, 564–568 (1982).
32. Strachan, T., Webb, D. A. & Dover, G. A. *EMBO J.* **4**, 1701–1708 (1985).
33. Dover, G. A. & Flavell, R. B. *Cell* **38**, 623–624 (1984).
34. Ohta, T. & Dover, G. A. *Genetics* **108**, 501–528 (1984).
35. Kneale, G. G. & Kennard, O. *Biochem. Soc. Trans.* **12**, 1011–1014 (1984).
36. Kost, T. A. *et al. Nucleic Acids Res.* **11**, 8287–8301 (1983).
37. Nudel, U. *et al. Nucleic Acids Res.* **11**, 1759–1771 (1983).
38. Hanukoglu, I. & Fuchs, E. *Cell* **33**, 915–924 (1983).
39. Hadjiolov, A. A. *et al. Nucleic Acids Res.* **12**, 3677–3583 (1984).
40. Chan, Y. L., Olvera, J. & Wool, I. G. *Nucleic Acids Res.* **11**, 7819–7831 (1983).
41. Dover, G. A. *Trends Genet.* **2**, 159–165 (1986).

Nuclear factor III, a novel sequence-specific DNA-binding protein from HeLa cells stimulating adenovirus DNA replication

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Dissection and reconstitution of the adenovirus DNA replication machinery has led to the discovery of two HeLa nuclear proteins which are required in conjunction with three viral proteins^{1–4}. One of these, nuclear factor I (NF-I), recognizes an internal region of the origin between nucleotides 25 and 40 (refs 4–8) and by binding to one side of the helix stimulates the initiation reaction up to 30-fold. NFI-binding sites have been observed upstream of several cellular genes, such as chicken lysozyme^{9,10}, human IgM¹¹ and human c-myc¹², and coincide in most cases with DNase I hypersensitive regions. Here we report the identification of a novel DNA-binding protein from HeLa nuclei, designated NF-III, that recognizes a sequence in the adenovirus origin very close to the NFI-binding site, between nucleotides 36 and 54. This sequence includes the partially conserved nucleotides TATGATAATGAG. NF-III stimulates DNA replication four- to sixfold by increasing the initiation efficiency. Potential cellular binding sites include promoter elements of the histone H2B gene, the human interferon β gene, the human and mouse immunoglobulin V_{κ} and V_{H} genes and the mammal/chicken/*Xenopus laevis* U1 and U2 small nuclear RNA genes. Furthermore, a subset of the herpes simplex virus immediate early promoter specific TAATGARAT elements is homologous with the adenovirus 2 (Ad-2) NFIII-binding site.

Replication of adenovirus DNA in human cells occurs by a protein-priming mechanism in which a precursor to the viral terminal protein (pTP) becomes covalently bound to the first nucleotide, a dCMP residue. The 3'-OH group of dCMP in this

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1. Hamada, H., Petrino, M. G. & Kakunaga, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6465–6469 (1982).

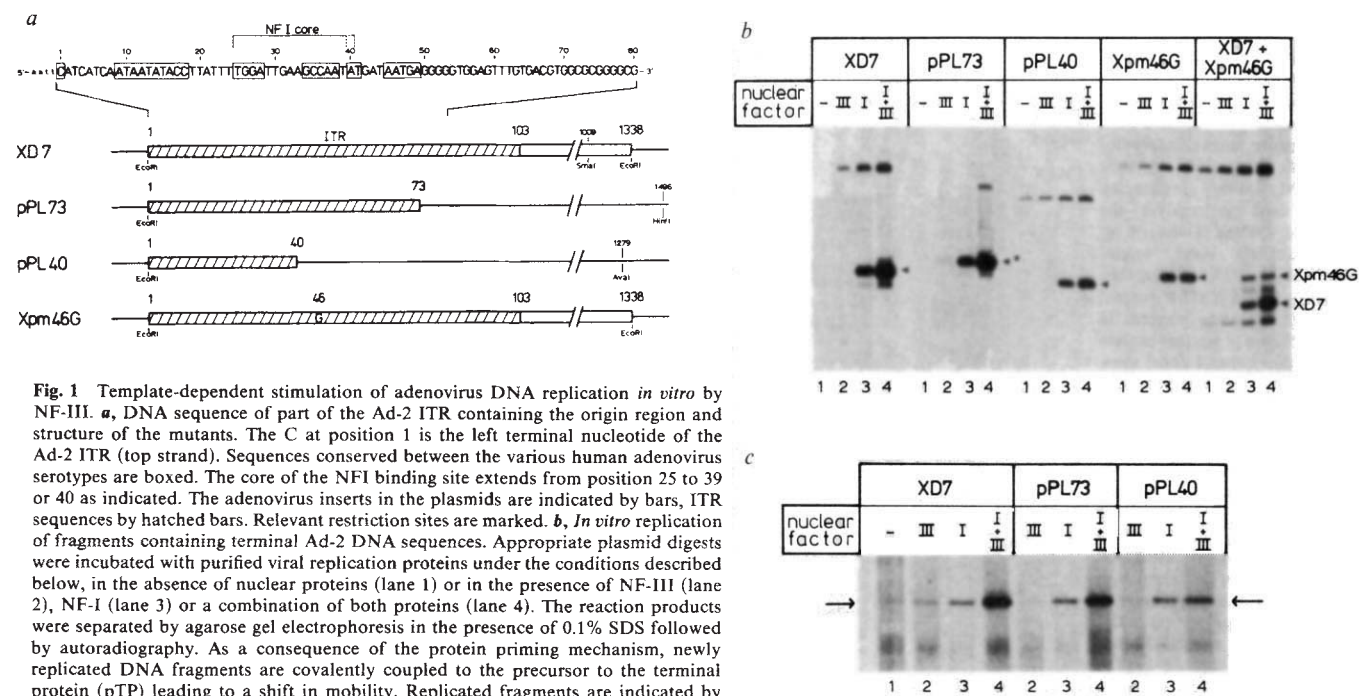


Fig. 1 Template-dependent stimulation of adenovirus DNA replication *in vitro* by NF-III. **a**, DNA sequence of part of the Ad-2 ITR containing the origin region and structure of the mutants. The C at position 1 is the left terminal nucleotide of the Ad-2 ITR (top strand). Sequences conserved between the various human adenovirus serotypes are boxed. The core of the NF1 binding site extends from position 25 to 39 or 40 as indicated. The adenovirus inserts in the plasmids are indicated by bars, ITR sequences by hatched bars. Relevant restriction sites are marked. **b**, *In vitro* replication of fragments containing terminal Ad-2 DNA sequences. Appropriate plasmid digests were incubated with purified viral replication proteins under the conditions described below, in the absence of nuclear proteins (lane 1) or in the presence of NF-III (lane 2), NF-I (lane 3) or a combination of both proteins (lane 4). The reaction products were separated by agarose gel electrophoresis in the presence of 0.1% SDS followed by autoradiography. As a consequence of the protein priming mechanism, newly replicated DNA fragments are covalently coupled to the precursor to the terminal protein (pTP) leading to a shift in mobility. Replicated fragments are indicated by arrowheads. **c**, Initiation of adenovirus DNA replication stimulated by NF-III. A digest of XD7, pPL73 or pPL40 was incubated as described in **b**, but in the presence of [α - 32 P]dCTP as the only nucleotide triphosphate. The products were analysed for the formation of the pTP-dCMP initiation product by SDS-polyacrylamide gel electrophoresis and autoradiography. The arrow indicates the position of pTP-dCMP.

Methods. The constructions of XD7 (ref. 28), pPL73 (ref. 17), pPL40 (ref. 7), and Xpm46G (ref. 8) have been described. XD7 was digested with *EcoRI*, pPL73 with *EcoRI* and *HinI*, pPL40 with *EcoRI* and *AvaI* and Xpm46G with *EcoRI*, while for the replication of a mixture of XD7 and Xpm46G, XD7 was also digested with *SmaI*. DNA replication assays were performed in a final volume of 15 μ l containing 25 mM Hepes-KOH (pH 7.5), 4 mM MgCl₂, 0.4 mM DTT, 1.7 mM ATP, 5 mM creatine phosphate, 5 μ g ml⁻¹ creatine kinase, 17 μ M each of dATP, dGTP and dTTP, 2 μ M [α - 32 P]dCTP (25 Ci mmol⁻¹), 2 mU pTP-adenovirus DNA polymerase complex, 1.25 μ g adenovirus DNA-binding protein, 1 μ l HeLa cytosol, 0.6 mM aphidicolin, 30 ng XD7 or 14 ng pPL73 or 22 ng pPL40 or 30 ng Xpm46G or 15 ng XD7 and 15 ng Xpm46G and 1 μ l NF-I or NF-III when indicated. The amounts of plasmid DNA were chosen to obtain identical concentrations of origin fragments. Reaction mixtures were incubated for 60 min at 37 °C. When assaying initiation dATP, dGTP and dTTP were omitted⁸. The purification of pTP-DNA polymerase²⁹, DNA-binding protein³⁰, cytosol³¹ and NF-I⁷ has been described. For NF-I hydroxylapatite fractions were used. NF-III was purified as follows. Nuclei from 10¹⁰ HeLa cells were isolated³² and washed three times with 25 mM Tris-HCl (pH 7.5), 10% sucrose, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 20 μ g ml⁻¹ L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK). The nuclei were suspended in the same buffer containing 1 mM EDTA and 1 mM dithiothreitol (DTT). Nuclear proteins were extracted by addition of 5 M NaCl to 0.3 M final concentration and slow stirring at 0 °C for 30 min. Cellular debris was removed by successive centrifugations at 12,000 g for 20 min and 105,000 g for 40 min. The final supernatant was adjusted to 0.2 M NaCl by addition of buffer A (25 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 20 μ g ml⁻¹ TPCK, 20% glycerol) and applied to a DEAE-cellulose column equilibrated with buffer A containing 0.2 M NaCl. The flow-through was dialysed against buffer B (25 mM Tris-HCl pH 7.5, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 20 μ g ml⁻¹ TPCK, 20% glycerol, 10% sucrose, 0.01% Nonidet P40) containing 0.03 M NaCl and applied to a second DEAE-cellulose column equilibrated with buffer B containing 0.03 M NaCl. The column was washed with buffer B-0.03 M NaCl and eluted with a linear gradient of 0.03–0.3 M NaCl in buffer B. The peak of NF-III activity was present in the first wash fractions following the flow-through. These fractions were applied to a phosphocellulose column equilibrated with buffer B-0.03 M NaCl. Bound material was eluted with a linear gradient of 0.03–1 M NaCl in buffer B. NF-III activity eluted at 0.15 M NaCl. NF-III-containing fractions were combined, adjusted to 0.05 M NaCl by addition of buffer B and applied to a denatured DNA-cellulose column equilibrated with buffer B-0.05 M NaCl. Bound material was eluted with a linear gradient of 0.05–1 M NaCl in buffer B. NF-III activity eluted at 0.24 M NaCl. All purification steps were performed on ice or at 4 °C. Throughout the purification, NF-III activity was monitored by its ability to stimulate the replication of the origin containing 6146 and 5641 bp *XhoI* B and C fragments, respectively, obtained from the Ad-5 DNA-TP complex¹⁴.

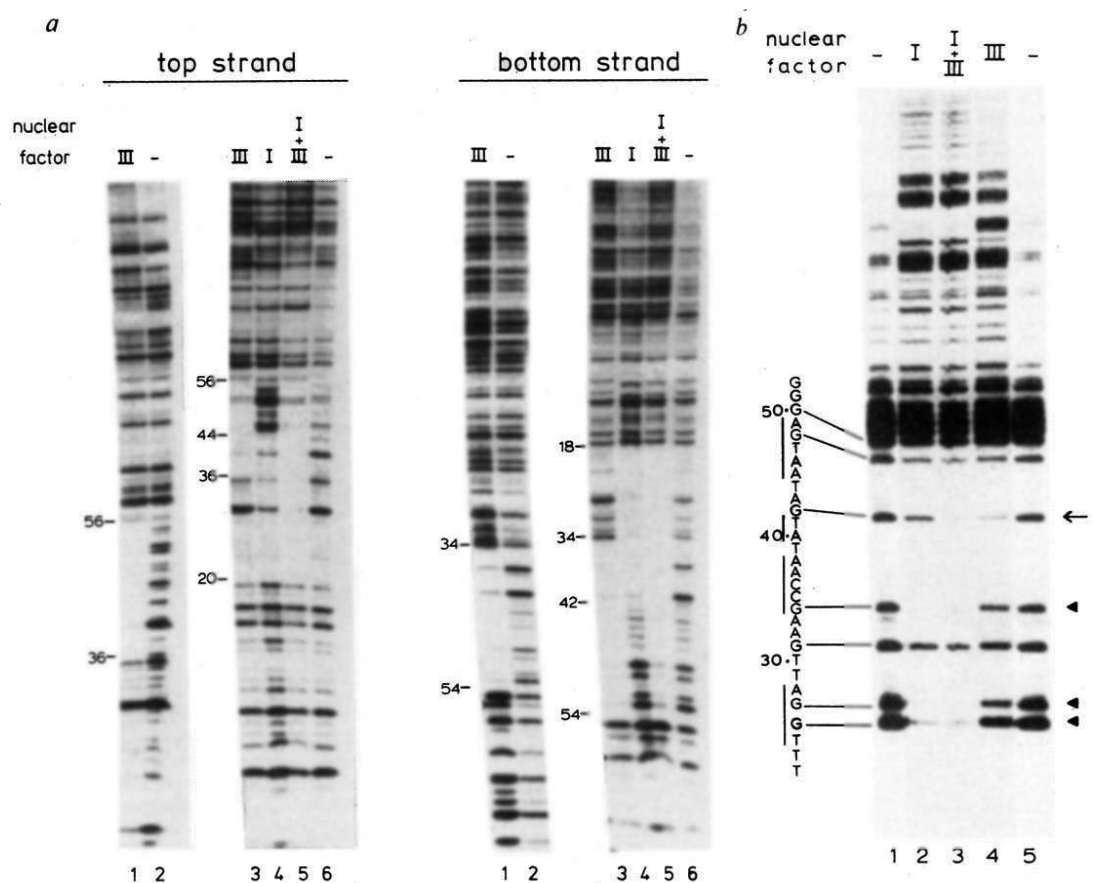
pTP-dCMP complex serves as a primer for further polymerization by a strand displacement mechanism (for reviews, see refs 1, 2, 13). Both initiation and elongation can be accomplished *in vitro* using five purified proteins. Three of these are encoded by the virus: the single-strand DNA-binding protein (DBP), DNA polymerase (pol) and pTP. The remaining proteins, NF-I and -II, are purified from HeLa nuclei^{3,4}. Previously we observed that sera from patients suffering from various autoimmune diseases inhibit adenovirus DNA replication *in vitro*¹⁴. This inhibition was only observed when crude HeLa nuclear extracts were used, but not in the presence of purified NF-I. As NF-II was not required in the assay, this suggested the existence of additional HeLa proteins required for optimal replication. Therefore, we searched directly for such proteins using a replication assay containing DBP, pTP, pol and NF-I. HeLa nuclei were extracted with 0.3 M NaCl and the extract was fractionated on DEAE-cellulose. Screening of the column fractions revealed the presence of a new protein that stimulated replication four- to sixfold. This protein, NF-III, eluted at 30 mM NaCl whereas NF-I eluted at 80 mM NaCl. NF-III was further purified by phosphocellulose and denatured DNA cellulose chromatography. The

stimulatory activity of NF-III was sensitive to 0.1 mM *N*-ethylmaleimide and was 90% inactivated by heat treatment for 15 min at 50 °C.

To determine whether specific regions of the Ad-2 inverted terminal repeat (ITR) were required for stimulation by NF-III we used plasmid DNA containing either the wild-type origin (XD7) or several mutant origins. Origin-containing plasmid DNA can replicate *in vitro* provided that the DNA is linearized at the origin^{15,16}. Protein-primed replication is visualized by a shift in electrophoretic mobility of the origin fragment caused by covalent binding of the pTP to the synthesized DNA. The plasmids pPL73 and pPL40 contain the terminal 73 and 40 base pairs (bp) of the Ad-2 ITR, respectively (Fig. 1a). Xpm46G is a derivative of XD7 with an $\text{A} \rightarrow \text{G}$ transition at position 46.

Replication of all these mutants was stimulated by NF-I similar to wild type (Fig. 1b) in accordance with previous results^{7,8,17}. However, only wild-type XD7 and pPL73 were stimulated by NF-III, whereas pPL40 and Xpm46G were not. Similar results were obtained when the initiation reaction (pTP-dCMP synthesis) was studied (Fig. 1c). This indicates that NF-III, like NF-I, exerts its effect on the level of initiation. But

Fig. 2 Localization of the NF-III binding site in the Ad-2 ITR. **a**, DNase I footprint analysis of NF-III- and NF-I-binding sites in the Ad-2 ITR. The figure shows autoradiographic exposures of 10% polyacrylamide sequencing gels used to analyse the partial DNase I digests of end-labelled Ad-2 origin-containing DNA fragments. Lanes 2 and 6 show control DNase digestion patterns in the absence of nuclear protein fractions. Lanes 1 and 3 show the DNase cleavage pattern in the presence of NF-III. The remaining lanes show the DNase digestion patterns in the presence of NF-I (lane 4) or both NF-I and NF-III (lane 5). Numbers, positions in the Ad-2 ITR as in Fig. 1a, which were determined using G-tracks and specific restriction fragments as markers. **b**, Methylation protection analysis of NF-III and NF-I binding sites in the Ad-2 ITR. Autoradiographic exposure of a 10% polyacrylamide sequencing gel used to analyse the partially methylated end-labelled Ad2 origin containing DNA fragments after cleavage by piperidine treatment. Lanes 1 and 5 show control methylation patterns in the absence of nuclear protein fractions. Lanes 2 and 4 show the methylation pattern obtained in the presence of NF-I and NF-III, respectively, while lane 3 shows the methylation pattern obtained in the presence of both NF-I and NF-III. The sequence at the left of the autoradiogram shows part of the Ad-2 ITR (top strand) and indicates the position of primary methylation sites. The G-residue protected by NF-III is indicated with an arrow, while residues protected by NF-I are indicated by arrowheads.



the sequence at the left of the autoradiogram shows part of the Ad-2 ITR (top strand) and indicates the position of primary methylation sites. The G-residue protected by NF-III is indicated with an arrow, while residues protected by NF-I are indicated by arrowheads.

Methods. The footprint and methylation protection probes were constructed as follows: To the single-stranded M13 clones MXE-1 and MXE-4, 5' end-labelled synthetic oligonucleotides were hybridized. MXE-1 (ref. 8) and MXE-4 contain the top strand and the bottom strand of the EcoRI fragment of XD7, respectively. For top strand analyses the oligonucleotide was complementary to a M13 sequence in MXE-4 ranging from position -21 to -5 relative to position 1 of the Ad-2 insert. For bottom strand analyses the oligonucleotide was complementary to the Ad-2 sequence in MXE-1 ranging from position 65 to 81 of the Ad-2 insert. After hybridization the oligonucleotide primers were elongated with Klenow DNA polymerase⁸ giving the double-stranded constructs containing the Ad2 origin, 5' end-labelled at either the top (position -21) or the bottom strand (position 81). These constructs were incubated with 15 μ l NF-III (denatured DNA-cellulose fraction, see legend to Fig. 1) and/or 10 μ l NF-I (hydroxyl apatite fraction) in a total volume of 75 μ l containing 25 mM Hepes-NaOH (pH 7.5), 1 mM DTT, 0.5 mM MgCl₂, 50 mM NaCl, 50 ng pBR322, and 5 μ g bovine serum albumin for 60 min at 0°C. For DNase I footprints 5 μ l 50 mM MgCl₂ and 0.1 U DNase I were added. Digestion was allowed for 90 s at 24°C and stopped by addition of 4 μ l 0.2 M EDTA, 10% SDS. For methylation protection analyses 200 μ l sodium cacodylate (pH 8.0), 1 mM EDTA, and 0.5 μ l dimethyl sulphate were added. After 1 min at room temperature methylation was stopped by addition of 50 μ l 1.5 M sodium acetate, 50 mM, 1 M β -mercaptoethanol. After ethanol precipitation the DNA was dissolved in 100 μ l 1 M piperidine, incubated at 90°C for 30 min and analysed as described above.

stimulation by NF-III is dependent on the presence of sequences between 40 and 73, outside the recognition sequence of NF-I. Furthermore, a point mutation at position 46, located in a highly conserved pentanucleotide sequence ⁴⁵AATGA⁴⁹, completely prevents stimulation by NF-III. This suggests that NF-III acts by binding to sequences between 40 and 73 including ⁴⁵AATGA⁴⁹. Specific binding to origin fragments could indeed be demonstrated using a nitrocellulose-filter binding assay or an exonuclease III protection assay (data not shown).

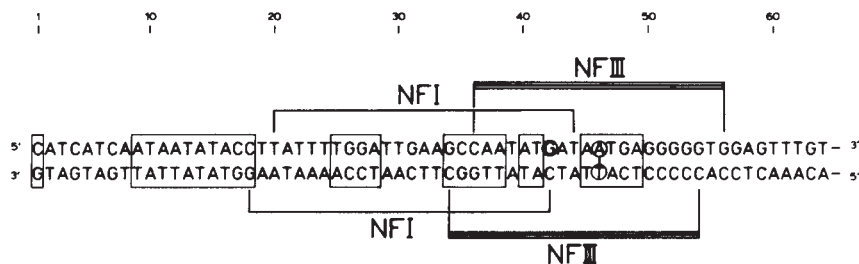
To identify the sequences involved in binding, we determined the region of the Ad-2 ITR that is protected against DNase I by footprint analysis. The Ad-2 origin containing region of the single-stranded M13 clones MXE-1 and MXE-4 were converted to double-stranded DNA by hybridization to 5' end-labelled synthetic oligonucleotides and elongation of these oligonucleotides using Klenow DNA polymerase. These constructs were partially digested with DNase I in the presence or the absence of NF-III (Fig. 2a, lanes 1, 2). NF-III protected the region between positions 36 and 56 of the top strand and the region between positions 34 and 54 of the bottom strand. Figure 2a also shows that the NF-III footprint partially overlaps the NF-I footprint (Fig. 2a, lanes 3, 4) and that mixtures of NF-I and NF-III protected the combined regions (Fig. 2a, lane 5). This

indicates that NF-I and NF-III can simultaneously bind to their recognition sequence and do not exclude each other.

Methylation protection experiments were performed to analyse possible contact points between NF-I and NF-III and their recognition sequence (Fig. 2b). NF-I protected G-residues at positions 26, 27 and 34 in the top strand from methylation by dimethylsulphate, whereas NF-III protected only the G-residue at position 42. Surprisingly, the conserved G-residue at position 48 was not a contact point. As guanines are methylated at the N⁷ position located in the major groove this suggests that, like NF-I, NF-III binds at least in part in the major groove of the DNA helix. Mixtures of NF-III and NF-I protected all four G-residues (Fig. 2b, lane 3) confirming that NF-I and NF-III can simultaneously bind to their recognition sequence.

The results are summarized in Fig. 3. The DNase I footprint of NF-III partially overlaps both the NF-I footprint and the NF-I core recognition sequence (Fig. 1a). This close spacing of the two proteins is remarkable. Using extensive contact point analysis we have recently shown that NF-I binds to one side of the helix (E. de Vries *et al.*, in preparation). The presence of a contact point between NF-III and a G-residue at position 42, only 6 bp removed from a contact point between NF-I and a G-residue at position 36, suggests that NF-III could bind at

Fig. 3 NF-III binding site in the adenovirus-2 origin. Highly conserved nucleotides within the terminal part of the Ad-2 ITR are boxed. Large brackets indicate the regions of both strands that are protected against DNase I digestion by either NF-I or NF-III. Circles, the G-residue protected against methylation by NF-III binding and the AT base pair essential for NF-III stimulation of replication.



least in part at the opposite side of the double helix compared to NF-I. At present we do not know whether the two proteins interact physically at their binding site. So far, we have not observed protein-protein interactions in solution between NF-I and NF-III during purification, comparable to, for example, the pTP-pol complex¹⁸.

The region protected by NF-III contains in its centre the sequence ³⁹TATGATAATGAG⁵⁰, much of which is conserved among the human and simian adenoviruses. A computer search reveals several homologous sequences in cellular DNA. A striking similarity was found with a promoter element of histone H2B genes, the H2B-specific homology block GTATGCAAATGAG, located close to the TATA box¹⁹. Several conserved promoter elements of other genes display similar homologous sequences. These genes include the human and mouse immunoglobulin V_K and V_H genes²⁰ (consensus ATGCAAATNA, ~100 nucleotides upstream of the start of the coding region), the human interferon β gene²¹ (ATGTAAATGA, about 100 nucleotides upstream of the transcription start site) and the mammal/chicken/*Xenopus laevis* U1 and U2 snRNA genes^{22,23} (consensus PyATGPyAPuATG located 250–220 nucleotides upstream from the 5' terminus of mature RNA). Furthermore, some TAATGARAT elements of the herpes simplex virus immediate early promoters²⁴ are highly homologous with the Ad-2 NF-III-binding site (consensus PuPyGNTAATGAPuATNC). It would be interesting to see whether NF-III binds to any of these regulatory elements.

The requirement for a functional NF-I site for adenovirus DNA replication *in vivo* has been established^{25,26}, but the role of NF-III is unclear. Plasmids containing either nucleotides 1–45 or 1–67 are reported to be fully active in replication *in vivo*^{25,26}. Interestingly, replication-competent Ad-4 DNA which lacks a functional NF-I site²⁷ binds NF-III efficiently in filter binding assays (G.J.M.P., A.M.A.A. Kavelaars, and P.C. van der V., unpublished observations). Recently, we were informed that a protein with similar properties to NF-III has been found by P. J. Rosenfeld and T. J. Kelly (personal communication).

Since submission of this manuscript, in collaboration with Drs G. Tebb, D. Bohmann and I. W. Mattaj (EMBL, Heidelberg) we have shown by footprint analysis that NF-III binds well to the *Xenopus laevis* U2 upstream conserved sequence (–269 to –259). This sequence is required for efficient transcription of U2 snRNA genes (D. Bohmann, T. Dale, G. Tebb, H. R. Schöler, I. W. Mattaj & W. Keller, in preparation) suggesting that NF-III can function both in DNA replication and transcription.

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1. Challberg, M. D. & Kelly, T. J. *A. Rev. Biochem.* **51**, 907–934 (1982).
2. Sussenbach, J. S. & Van der Vliet, P. C. *Curr. Top. Microbiol. Immun.* **109**, 53–73 (1983).
3. Nagata, K., Guggenheimer, R. A., Enomoto, T., Lichy, J. H. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6438–6442 (1982).
4. Nagata, K., Guggenheimer, R. A. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4266–4270 (1983).
5. Rawlins, D. R., Rosenfeld, P. J., Wides, R. J., Challberg, M. D. & Kelly, T. J. *Cell* **37**, 309–319 (1984).
6. Guggenheimer, R. A., Stillman, B. W., Nagata, K., Tamanoi, F. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3069–3073 (1984).

7. Leegwater, P. A. J., Van Driel, W. & Van der Vliet, P. C. *EMBO J.* **4**, 1515–1521 (1985).
8. De Vries, E., Van Driel, W., Tromp, M., Van Boom, J. H. & Van der Vliet, P. C. *Nucleic Acids Res.* **13**, 4935–4952 (1985).
9. Borgmeyer, U., Nowock, J. & Sippel, A. E. *Nucleic Acids Res.* **12**, 4295–4311 (1984).
10. Nowock, J., Borgmeyer, U., Püschel, A. W., Rupp, R. A. W. & Sippel, A. E. *Nucleic Acids Res.* **13**, 2045–2061 (1985).
11. Hennighausen, L. *et al. Nature* **314**, 289–292 (1985).
12. Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. *Cell* **37**, 381–391 (1984).
13. Stillman, B. W. *Cell* **35**, 7–9 (1983).
14. Puijn, G. J. M., Kusters, H. G., Gmelig Meyling, F. H. J. & Van der Vliet, P. C. *Eur. J. Biochem.* **154**, 363–370 (1986).
15. Tamanoi, F. & Stillman, B. W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2221–2225 (1982).
16. Van Bergen, B. G. M., Van der Ley, P. A., Van Driel, W., Van Mansfeld, A. D. M. & Van der Vliet, P. C. *Nucleic Acids Res.* **11**, 1975–1989 (1983).
17. Leegwater, P. A. J., Van der Vliet, P. C., Rupp, R. A. W., Nowock, J. & Sippel, A. E. *EMBO J.* **5**, 381–386 (1986).
18. Enomoto, T., Lichy, J. H., Ikeda, J.-E. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6779–6783 (1981).
19. Harvey, R. P., Robins, A. J. & Wells, J. R. E. *Nucleic Acids Res.* **10**, 7851–7863 (1982).
20. Falkner, F. G. & Zachau, H. G. *Nature* **310**, 71–74 (1984).
21. Fujita, T., Ohno, S., Yasumitsu, H. & Taniguchi, T. *Cell* **41**, 489–496 (1985).
22. Krol, A., Lund, E. & Dahlberg, J. E. *EMBO J.* **4**, 1529–1535 (1985).
23. Ciliberto, G., Buckland, R., Cortese, R. & Philipson, L. *EMBO J.* **4**, 1537–1543 (1985).
24. Whitton, J. L. & Clements, J. B. *Nucleic Acids Res.* **12**, 2061–2079 (1984).
25. Hay, R. T. *EMBO J.* **4**, 421–426 (1985).
26. Wang, K. & Pearson, G. D. *Nucleic Acids Res.* **13**, 5173–5187 (1985).
27. Hay, R. T. *J. molec. Biol.* **186**, 129–136 (1985).
28. Pearson, G. D. *et al. Gene* **23**, 293–305 (1983).
29. Rijnders, A. W. M., Van Bergen, B. G. M., Van der Vliet, P. C. & Sussenbach, J. S. *Nucleic Acids Res.* **11**, 8777–8789 (1983).
30. Tsernoglou, D., Tucker, A. D. & Van der Vliet, P. C. *J. molec. Biol.* **172**, 237–239 (1984).
31. Van der Vliet, P. C., Van Dam, D. & Kwant, M. M. *FEBS Lett.* **171**, 5–8 (1984).
32. Challberg, M. D. & Kelly, T. J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 655–659 (1979).

Green light-mediated photomorphogenesis in a dinoflagellate resting cyst

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Developmental responses to light are well known and ubiquitous among higher plants¹, but examples of such responses among the algae are less common. Those responses which have been reported in this group are generally photoperiodic or require prolonged light exposures; most involve macrophytes². We now report a non-photosynthetic, low-threshold, photomorphogenic response in a unicellular alga, the dinoflagellate *Scrippsiella trochoidea* (Stein) Loeblich. In contrast to the reported behaviour of most other dinoflagellate species, resting cysts of *S. trochoidea* require light to germinate. This requirement is satisfied to a large extent by low photon fluences delivered in exposures as short as 1 second. Green light is most effective in eliciting the response. Given the importance of dinoflagellates as primary producers in many aquatic ecosystems and the potential role of resting cysts in controlling the dynamics of natural dinoflagellate populations, the present observations are of obvious ecological significance. The primitive phylogenetic standing of dinoflagellates³, and the relative rarity of green light-mediated photomorphogenic responses in eukaryotes generally^{4,5}, suggest that this phenomenon may also hold considerable evolutionary and photophysiological interest.

Temperature has been almost universally cited as the environmental factor exerting primary control over germination in dinoflagellate resting cysts^{6,7}. When considered, light conditions have generally been found to exert little effect on cyst germination^{8–11}.

In the two studies to date which have demonstrated a light effect, germination was delayed but not prevented by darkness^{12,13}. Preliminary studies with *S. trochoidea* have confirmed that temperature is important in controlling germination in cysts of this species, but have also demonstrated that germination may be significantly reduced in the absence of light¹⁴. The present study was undertaken to elucidate the influence of light on germination in cysts of *S. trochoidea*.

Scripsiella trochoidea is a small photosynthetic marine dinoflagellate of widespread neritic distribution. Cysts for the present study were produced in axenic clonal cultures (clone SA10, from Perch Pond, Falmouth, Massachusetts) under a 14:10 h daily light:dark (L:D) cycle at 18 °C¹⁴. Within a week of the appearance of cysts, the cultures were enriched with inorganic nutrients at $f/2$ levels¹⁵ and placed in darkness at 3 or 18 °C. Every precaution was taken to insure that no further light reached these cultures. Sampling and manipulations, when necessary, were carried out in total darkness.

Under these strictly dark conditions, cysts stored at 18 °C failed to germinate over the 120 days of the experiment, although conditions should otherwise have been optimal for excystment (Fig. 1). Exposure of these cysts to the standard 14:10 h L:D cycle ($\sim 650 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), at the same temperature, resulted in rapid germination.

An upward shift in temperature commonly results in rapid and complete germination of dinoflagellate cysts which have been stored at low temperature (Fig. 1)^{6,7}. In the present case, however, no germination occurred among cysts so treated if they were deprived of all light (Fig. 2). In contrast, cysts exposed to as little as 2 min of light germinated rapidly and only slightly less successfully (though significantly so) than those exposed to the same level of illumination for 14 h daily ($P < 0.001$). The response to 60 min of light was indistinguishable from that in the 2-min treatment.

The relationship between total 'white light' photon fluence and germination underscores the sensitivity of *S. trochoidea* cysts to low levels of light (Fig. 3). A 50% response (based on a maximum achieved germination frequency of 60%) occurs in these cysts at approximately $0.2 \mu\text{mol m}^{-2}$ photon fluence. This photon fluence corresponds to an exposure time at standard culturing irradiances (see above) of far less than 1 s.

The germination response appears to be dependent upon photon fluence ($\mu\text{mol m}^{-2}$), rather than fluence rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) or exposure time separately. Thus, equivalent germination is elicited by equal photon fluences, whether applied over 10 or 1,000 s (Fig. 4).

These results clearly demonstrate that light is required for germination in *S. trochoidea* cysts. Furthermore, the fact that a low-irradiance exposure lasting 1 s is sufficient to stimulate germination raises the possibility that previous studies with dinoflagellate cysts (including our own), designed primarily with longer-term light effects in mind, may have been unable to distinguish between such a low threshold response and true 'dark' germination.

The response by *S. trochoidea* cysts to the same photon fluence ($0.12 \mu\text{mol m}^{-2}$) at different wavelengths is shown in Fig. 4. Germination is maximal in the 550-nm (yellow-green) band, with wavelengths above 620 nm being ineffective at this fluence level. The response drops more slowly on the low side of 550 nm, and is still apparent in the blue (450 nm) band. There was no evidence of modification of the 550-nm band response by subsequent exposure to red or far-red light ($650 \pm 20 \text{ nm}$ or $750 \pm 20 \text{ nm}$) at equivalent fluence levels (data not shown). The relatively low germination frequency among all the wavelength-band treatments is consistent with the low response achieved in the white-light controls of this experiment (Fig. 4) and therefore cannot be taken to indicate a low response to monochromatic light generally. The cause of the reduced germination in this experiment is not known, but the pattern of response to different wavelength bands was confirmed by preliminary experiments

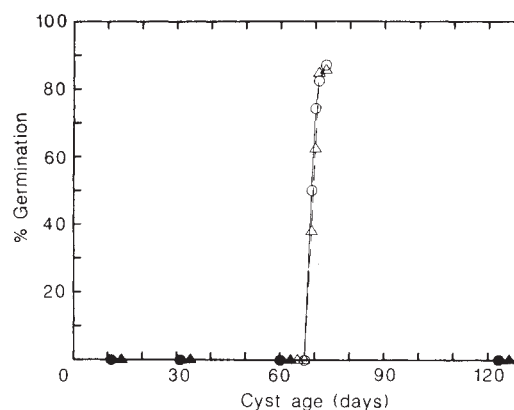


Fig. 1 Germination of *S. trochoidea* cysts placed in the dark at 3 °C (●) or 18 °C (▲) starting at age 7 days. On day 65, some cysts from both the 3 °C and 18 °C dark storage (○, △, respectively) were placed under a continuing 14:10 h L:D cycle at 18 °C.

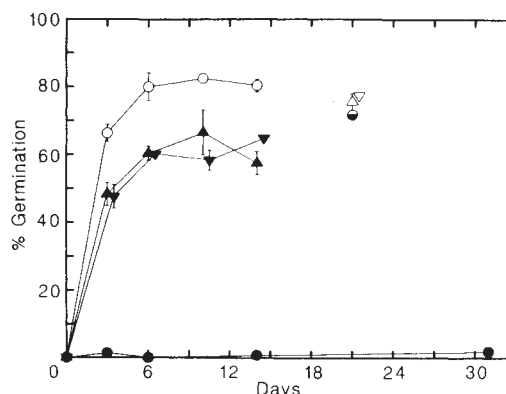


Fig. 2 Time course of germination by cysts (stored at 3 °C in the dark) on transfer to 18 °C and exposure to a continuing 14:10 h L:D cycle (○), to 60 min of light only (▼), to 2 min of light (▲), and to no light (●). On day 14, cysts from the three latter treatments were exposed to the continuing 14:10 h L:D cycle and assessed 6 days later (▽, △, ●, respectively); means ± 1 s.e. ($n = 2$).

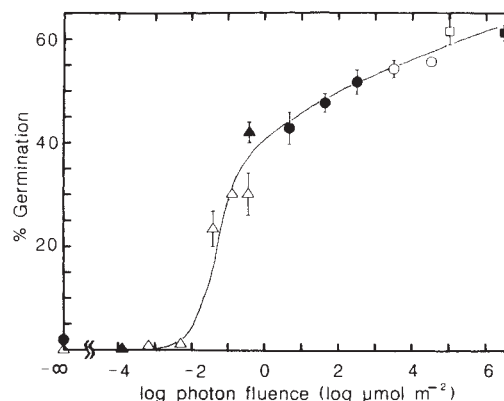


Fig. 3 Effect of 'white light' photon fluence ($\mu\text{mol m}^{-2}$) on germination frequency. Incandescent source, except □ and ■ (which are taken from Fig. 2), illuminated with a cool white fluorescent source. Photon fluence is the product of exposure time and fluence rate [measured for photosynthetically active wavelengths only ($400 \text{ nm} < \lambda < 700 \text{ nm}$) with a scalar irradiance meter (Biospherical Instruments, Inc.) and adjusted, as necessary, with neutral density filters]. Exposure times are ▲, 1 s; △, 5 s; ●, 12 s; □, 120 s; ■, 3,600 s; means ± 1 s.e. ($n = 3$, except $n = 6$ for □ and ■). Line drawn by eye.

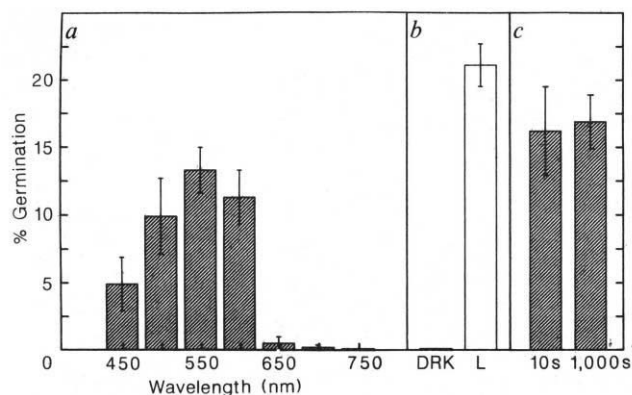


Fig. 4 Germination response of *S. trochoidea* cysts to wavelength. **a**, Bars show the frequency of germination achieved in cysts exposed to equal photon fluences ($\sim 0.12 \mu\text{mol m}^{-2}$) in seven different wavelength bands. Incandescent source, in combination with wide-band interference filters (40 nm half-power bandwidth, blocking outside of band better than 0.1% between low ultraviolet and 1,000 nm; Ditic Optics Inc.). Exposure time was 10 s in all cases; fluence rate was adjusted with neutral density filters and by varying source voltage; means ± 1 s.e. ($n = 3$); bar width represents half-power bandwidth, on wavelength scale shown. **b**, Open bar is the 'white light' control; unfiltered incandescent source, $4.2 \mu\text{mol m}^{-2}$ (corresponding to $\sim 0.5 \mu\text{mol m}^{-2}$ in the 550 ± 20 -nm band). DRK bar, the dark control. **c**, Germination response to approximately equivalent photon fluences ($\sim 1 \mu\text{mol m}^{-2}$, $\lambda = 550 \pm 20$ nm) administered over 10 s or 1,000 s, as indicated.

which, however, involved less complete coverage of the spectrum.

Overall, our data indicate that the response to light in *S. trochoidea* cysts is not photosynthetic. This conclusion is based on the low photon fluence requirement of the response and its relative sensitivity to green light as compared with blue or red. The conclusion is further supported by the inability of dark-stored *S. trochoidea* cysts to photosynthesize immediately after their transfer to light¹⁴.

Other non-photosynthetic responses to light which have been reported in dinoflagellates include phototaxis¹⁶⁻¹⁸ and growth inhibition by far-red exposures¹⁹. Generally, green wavelengths are not active in these phenomena; the photoreceptor systems responsible are therefore probably different from that involved in the light-triggered germination of *S. trochoidea* cysts.

Among algal resting stages generally, germination of cyanobacterial akinetes²⁰⁻²² and diatom resting spores^{23,24} has been widely found to be light-dependent. In contrast to the present case, the response in akinetes is maximal in the red wavelengths and generally requires extended exposures (hours to days) and high photon fluences^{21,25}. Similarly, diatom resting spores are responsive only to relatively high fluence rates²³. In neither of these cases can photosynthetic involvement be confidently discounted.

The extent to which the unique light requirement described here could control the germination of *S. trochoidea* cysts in the natural system depends on several factors, including the optical properties of overlying waters (note that coastal waters generally transmit maximally in the green band), the extent to which reciprocity in the response holds (that is, the maximum time over which 'photon counting' can occur), and the influence of cyst age and environmental parameters on the response itself. Preliminary calculations, based on conservative assumptions about these factors (including a maximum photon counting time of 1,000 s), indicate that in average coastal waters of moderate depth (~ 40 – 80 m) significant germination will occur, although the germination frequency ultimately achieved could be light limited¹⁴. On the other hand, in particularly deep or turbid waters, light could be sufficiently attenuated to prevent germination completely. Furthermore, the burial of cysts in the sediment

would probably result in prohibitively low light levels regardless of the depth of the overlying water column. In these latter cases, resuspension from the sediment surface and/or advection to shallower depths would presumably be required before significant germination could occur.

Although the ecological consequences of light-triggered germination in *S. trochoidea* cannot be fully appraised without further information on the physiology of the response, it is clear that light can no longer be ignored in considerations of the behaviour of dinoflagellate cysts in the natural system.

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- Shropshire, W. Jr & Mohr, H. (eds) *Encyclopedia of Plant Physiology*, new Ser. Vol. 16A, B (Springer, Berlin, 1983).
- Dring, M. J. & Luning, K. in *Encyclopedia of Plant Physiology*, new Ser. Vol. 16B (eds Shropshire, W. Jr & Mohr, H.) 545–568 (Springer, Berlin, 1983).
- Dodge, J. D. *Br. phycol. J.* **18**, 335–356 (1983).
- Klein, R. M. *Pl. Physiol.* **63**, 114–116 (1979).
- Tanada, T. *Physiol. Pl.* **58**, 475–478 (1983).
- Dale, B. in *Survival Strategies of the Algae* (ed. Fryxell, G. A.) 69–136 (Cambridge University Press, 1983).
- Pfiester, L. A. & Anderson, D. M. in *The Biology of Dinoflagellates* (ed. Taylor, F. J. R.) (Blackwell Scientific, Oxford, in the press).
- Huber, G. & Nipkow, F. *Flora* **116**, 114–215 (1923).
- Anderson, D. M. & Wall, D. J. *Phycol.* **14**, 224–234 (1978).
- Krupa, D. *Ekol. pol.* **29**, 545–570 (1981).
- Hall, S. thesis, Univ. Alaska (1982).
- Endo, T. & Nagata, H. *Bull. Plankt. Soc. Japan* **31**, 23–33 (1984).
- Anderson, D. M., Taylor, C. D. & Armbrust, E. V. *Limnol. Oceanogr.* (submitted).
- Binder, B. J. thesis, Massachusetts Inst. Technol. Woods Hole Oceanographic Institute WHOI-86-9 (1986).
- Guillard, R. R. L. & Ryther, J. H. *Can. J. Microbiol.* **8**, 229–239 (1962).
- Halldal, P. *Physiol. Pl.* **11**, 118–153 (1958).
- Hand, W. G., Forward, R. B. & Davenport, D. *Biol. Bull.* **133**, 150–165 (1967).
- Forward, R. B. *Jr. Planta* **92**, 248–258 (1970).
- Lipps, M. J. *J. Phycol.* **9**, 237–242 (1973).
- Yamamoto, Y. *J. gen. appl. Microbiol.* **22**, 311–323 (1976).
- Braune, W. *Arch. Microbiol.* **122**, 289–295 (1979).
- Chauvat, F., Corre, B., Herdman, M. & Joset-Espardellier, F. *Arch. Microbiol.* **133**, 44–49 (1982).
- Hollibaugh, J. T., Siebert, D. L. R. & Thomas, W. H. *J. Phycol.* **17**, 1–9 (1981).
- Hargraves, P. E. & French, F. W. in *Survival Strategies of the Algae* (ed. Fryxell, G. A.) 49–68 (Cambridge University Press, 1983).
- Reddy, P. M. & Talpasayi, E. R. S. *Biochimie Physiologie Pflanzen* **176**, 105–107 (1981).

The crystal structure of d(GGATGGGAG) forms an essential part of the binding site for transcription factor IIIA

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Most genes in higher organisms are activated by the binding of proteins called transcription factors. One such protein, transcription factor IIIA (TFIIIA) from the frog, activates the gene for 5S RNA by binding to the region of the gene between nucleotides 45 and 97. This binding site has been defined by a variety of biochemical studies, including base-deletion experiments and DNase I footprinting¹. The protein also binds to the gene product: in immature frogs it is stored as a complex with 5S RNA. From the observation that TFIIIA can bind to either double-helical DNA or RNA, and from their own measurements, Rhodes and Klug² have proposed that the DNA-binding site for TFIIIA has an RNA-like structure. Here we present the crystal structure analysis of a part of the DNA-binding site (nucleotides 81–89 of the gene)

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which forms a particularly strong interaction with the protein, and show that it has a conformation similar to the A' form of double-helical RNA.

Miller *et al.*³ have shown recently that the amino-acid sequence of TFIIA contains multiple repeats of a 30-residue unit which they called a 'finger'. There are nine such fingers in the 344-residue TFIIA, each of which contains the sequence Cys...Cys...His...His and is thought to complex a zinc ion. Finger sequences have now been found in at least four other DNA-binding proteins from higher organisms^{2,4-6}, suggesting that this structural unit is of primary importance in many cell processes. It is not known definitively how much DNA each finger covers but assuming that there is one TFIIA protein bound per 50 base pairs (bp) of DNA⁷, each finger would have to recognize about 5.5 bp, or one-half of a double-helical turn. Indeed, Rhodes and Klug² have recently provided evidence for a structural repeat of 5.7 bp in the TFIIA binding site, to match the required amount of DNA per finger. At the suggestion of Dr A. Klug we have investigated the three-dimensional structure of part of the gene for 5S RNA by single-crystal X-ray diffraction methods. Nucleotides 81-89 of the gene were chosen because, as shown by methylation interference⁸, methylation protection⁹ and base deletion experiments^{10,11}, this stretch of DNA is more important for the binding of TFIIA than any other part. The base sequence of our selected molecule, and its relation to the length of DNA covered by TFIIA, are shown in Fig. 1.

The two strands d(GGATGGGAG) and d(CTCCCATCC) were synthesized by the triester method¹² and purified separately. They were then mixed in equimolar proportions, as calculated from their respective ultraviolet absorbances at 260 nm, to form a double helix of relative molecular mass 5,500. Crystals were grown by vapour diffusion against 6-20% 2-methyl-2,4-pentandiol (MPD) from a mixture which initially contained 0.2 mM DNA, 12 mM magnesium acetate, 12 mM sodium cacodylate (pH 6.5) and 0.8 mM spermine HCl. The crystals grew as tetragonal rods to a size of $0.5 \times 0.18 \times 0.18$ mm³ during 5 months at room temperature, and were stabilized by diffusion to 60% MPD. Precession photographs show that the space group is $P4_1$ or $P4_3$, with cell dimensions $a = b = 45.29$ Å, $c = 24.73$ Å. The unit cell contains eight strands, with one double helix of 9 bp in the asymmetric unit. A total of 1,786 reflections were measured between 25.0 and 2.7 Å on a diffractometer. These were corrected for the Lorentz factor, polarization and absorption effects, and then symmetry-equivalent reflections were averaged. Of the 1,459 unique reflections obtained, slightly fewer than 50% in the range 3.0-2.7 Å have intensities $I > \sigma(I)$. Thus, the resolution is approximately 3.0 Å.

The unit cell dimensions suggested that our crystal structure might be nearly isomorphous with those of d(CCGG) and d(GGCCGCC), both of which crystallize in the related space group $P4_32_12$ with 4 bp in the asymmetric unit^{13,14}. Hence, the starting coordinates for the 9-bp double helix in $P4_3$ were taken from our previously published model for poly(dG).poly(dC)¹⁵ and imposed on the coordinates of two d(CCGG) molecules in space group $P4_32_12$, with an appropriate shift in the origin. The model was refined against the X-ray data, first as a rigid body¹⁶ and then as a set of individual atoms connected by distance restraints¹⁷. Each atom in a base, sugar or phosphate group was assigned a temperature factor of 36, 41 or 46 Å², respectively, based on an overall temperature factor¹⁸ for the crystal of 40 Å². These were not refined further due to the limited number of observations. Many electron density maps were calculated to follow the progress of the refinement. No solvent molecules were included in the phasing model because the resolution was relatively low. After 80 cycles of refinement, the agreement, or 'R-factor', between measured and calculated structure factors for all 1,032 zero-sigma data in the range 10.0-3.0 Å is 33%. The R-factor for the same phasing model, but with the X-ray data weighted according to their observed standard deviations, is 18%. Further efforts to test the correctness of our structure

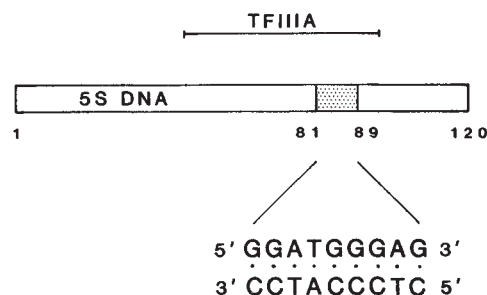


Fig. 1 A one-dimensional representation of the 120-bp coding-region for 5S RNA which shows the position of nucleotides 81-89 and the binding site for TFIIA⁷. The numbering scheme used places the start of transcription at nucleotide 1.

solution are described in the legend to Fig. 2.

The double helix formed by d(GGATGGGAG) and its complementary strand d(CTCCCATCC) is shown in Figs 2 and 3. The identity of the bases cannot be assigned with certainty at 3 Å resolution, as the contribution of the bases to the calculated X-ray intensities does not change very much when the molecule is turned upside-down, by rotating it about its central axis of approximate 2-fold symmetry. Nevertheless, the positions of the sugar-phosphate chains, which give the overall shape of the structure, are defined clearly by the electron density and, in either orientation of the molecule, these positions refine to closely similar fractional coordinates. Figure 2 shows a view of the molecule looking into the minor groove. This groove is shallow, but not flat, and its width (as measured by the distance between phosphorus atoms) varies from 15.7 Å near the bottom of Fig. 2 to 14.3 Å near the top. By way of comparison¹⁹, for example, fibre A-DNA has a minor-groove width of 17.0 Å, and fibre B-DNA, 11.7 Å.

Figure 3 shows a view of the molecule looking into the major groove. This groove is both deep and wide, the mean separation of phosphorus atoms being 14.8 Å (s.d. 0.1 Å). In the model for fibre A-DNA, the major groove width is 8.2 Å, and in the model for B-DNA, 17.0 Å. Along each strand, the distances between adjacent phosphates vary considerably about a mean of 5.9 (0.6) Å. Thus, the structure seen here is clearly distinct from models of DNA which are shown in textbooks.

The ways in which the base-pairs stack on each other are described directly by the parameters 'slide' and 'roll'²⁰. Slide is the distance by which the base pairs slip over each other in the direction of their long axes: in this structure the mean slide is 1.5 (0.7) Å. Roll, the angle by which adjacent base pairs open to the minor groove, averages 5 (13)°. The orientation of the base pairs can also be described in terms of parameters calculated relative to an imaginary helix axis. In our structure, the mean twist of base pairs with respect to this axis is 31.3 (9)°, yielding a loosely defined helical repeat of 11.5 bp. Other global parameters are the rise per residue of 3.0 (0.3) Å, a base-pair tilt of +10 (6)° and a displacement of base pairs from the central axis by 4.2 (0.3) Å. These averaged values are very close to those of the uniform model derived from the A' X-ray diffraction intensities of fibrous RNA^{21,22}. The model for A'-RNA has a helical repeat of 12.0 bp, a rise per residue of 3.0 Å, a base-pair tilt of +10° and a displacement of 4.5 Å.

Although some of the results from nuclear magnetic resonance experiments on DNA fragments in solution have been interpreted as being due to B-form structures, it is doubtful whether the technique can distinguish between the various global conformations of DNA such as 'A' and 'B'²³. However, as in the case of proteins, the three-dimensional structure of a DNA molecule in a highly hydrated crystal might indeed be the viable model for its stable conformation *in vivo*.

The results of our X-ray diffraction studies therefore agree with the proposal of Rhodes and Klug² that the DNA-binding

Fig. 2 Stereoscopic view of the structure of d(GGATGGGAG) and its complement d(CCCCCATCC), looking into the minor groove. Atoms P, C, N and O are represented by spheres of radii 1.9, 1.6, 1.5 and 1.4 Å, respectively; phosphate groups are shaded. In this view, the predominantly purine strand is on the left, with base G₈₁ at bottom. It is possible that the double helix packs in the crystal in a statistical fashion, using two orientations which are related to one another by a rotation of 180° about a line through the central base pair and perpendicular to the page. To assess the relative occupancies of these two possible orientations, the structure was refined independently in both settings. After 40 cycles in either case, the *R*-factor between measured and calculated structure factors (not weighted for noise) for all zero-sigma data to 3 Å was 33% for the model shown here, and 36% for the other orientation. Thus, it is not possible at present to determine which of the two orientations is preferred, but the one shown here agrees marginally better with the data. In both orientations, the cytosine base nearest to the crystallographic 4-fold screw axis (C₈₉ or C₈₁) is poorly defined in the electron density. As a check, we also analysed the structure using a real-space search program²⁸, choosing as models the coordinates of fibre B-DNA, fibre A-DNA¹⁹ and, as a control, those of the refined crystal structure. Searches were carried out in both *P*₄ and *P*₄₃. At low resolution, in the range 25–10 Å, the best solution for the B-model yields an *R*-factor of 40%, while both the A-model and the crystal coordinates yield a much lower 30%. At moderate resolution, in the range 10–5 Å, the best solution for the A-DNA fibre model yields 50% in both *P*₄ and *P*₄₃, whereas the refined crystal coordinates yield 22% in *P*₄₃, but 52% in *P*₄₁. These results confirmed the choice of space group as *P*₄₃, and further showed that the crystal X-ray structure is distinct from either the A or B forms of DNA found in pulled fibres^{19,29–32}.

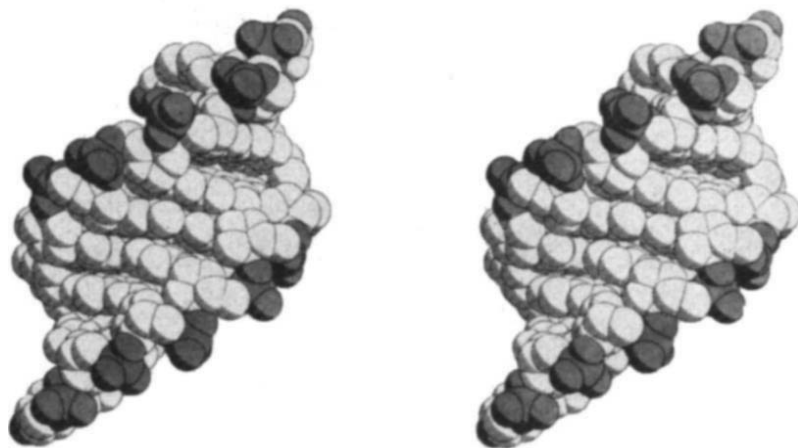
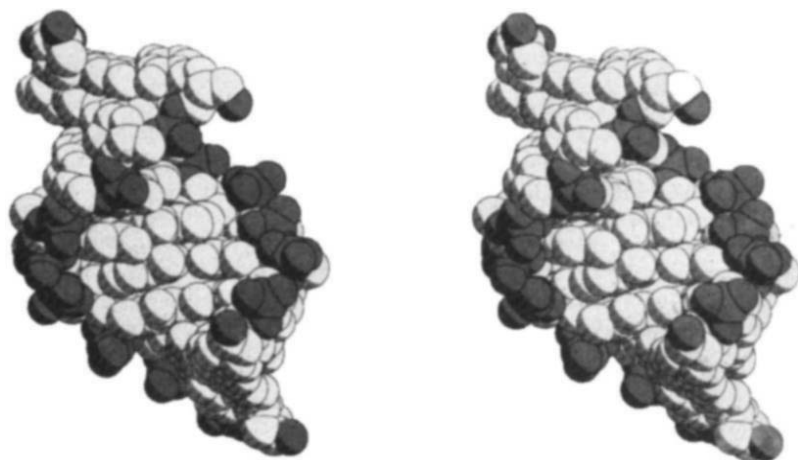


Fig. 3 Stereoscopic view of the structure showing the major groove (centre) and parts of the minor groove (top left and bottom right); the purine strand is on the right, with base G₈₁ at bottom. Based on methylation protection and nuclease digestion data, Fairall *et al.*⁹ have proposed a model for TFIID binding to 5S DNA in which the protein lies on one side of the double helix with its 'fingers' inserted every half-turn into the major groove. The fingers of the protein are hypothesized to fold as a twisted β -sheet³. Clearly, there is plenty of room for a twisted β -sheet to fit into the major groove shown in this figure. The ability of proteins to fit into the major groove of A-form double helices has been emphasized recently by Arnott³³. A further consequence of their model is that the protein residues between adjacent fingers would span the minor groove of the DNA, a distance which in our crystal structure is 15 Å. There is an invariant tyrosine or phenylalanine in these regions of the protein; this residue could lie in van der Waals contact with the exposed sugar rings of the minor groove, just as the terminal base pairs of DNA helices stack on the sugar residues of symmetry-related DNA molecules in this and many other structures^{13–15,26}. Huber and Wool³⁴ have studied the complex of TFIID with 5S RNA in solution. From their observation that the protein covers approximately the same region of bases in the RNA as in the DNA, they have also proposed that the DNA could have an RNA-like structure, at least when it is bound to the protein.



site of TFIID has an RNA-like structure, at least for nucleotides 81–89 in the crystal. What can be said about the conformation of this DNA in solution? Rhodes and Klug² have used Fourier methods on nuclease digestion data to show that the entire TFIID-binding site has an underlying structural repeat of 5.7 bp, or 11.4 bp in two repeats. Our 9-bp molecule is not long enough to show the characteristic variation in structure which accompanies this repeat. However, the local pattern of nuclease digestion for nucleotides 81–89 in solution² may be of interest. The enzyme DNase I does not cut well on either strand between nucleotides 82 and 83 (GpA = TpC), 84 and 85 (TpG = CpA), or 86 and 87 (GpG = CpC). These steps, in our best-refined model, are those which have extremes of local conformation such that they would be resistant to DNase I cleavage²⁴. The GpA step has a large positive roll of 27°, compared with a mean for the structure of 5°; the TpG step a very low twist of 16°, compared with a mean of 31°; and the GpG step has a high roll 17° and a high slide of 2.6 Å, compared with a mean of 1.5 Å.

Accompanying the periodicity in structure of the TFIID-binding site is a periodicity in sequence²: short runs of guanine

bases occur once every 5.6 bp. It seems likely that the preferred geometry of base stacking at the GpG step is the origin of an RNA-like structure for this region of DNA. Steps of the type GpG/CpC have now been observed in eight single-crystal X-ray structures: d(1CCGG)¹³, d(GGCCGGCC)¹⁴, d(GGGGCCCC)¹⁵, d(CCCCGGGG)^{14,25}, d(GGGCGCCC)²⁵, d(GGGATCCC) (U. Heinemann, unpublished results), d(GGTATACC)²⁶ and d(GGATGGGAG). In all of these, the five-membered ring of one guanine base lies above the six-membered ring of its neighbour. Such a local base-to-base overlap is characteristic of all A-form double helices: the sideways slippage of base pairs generates a double helix with a large hole in the centre when viewed from above^{15,20}.

Many other important DNA control regions are known to contain repeats of GpG². One example is the DNA-binding site for transcription factor Sp1, which contains repeats of the sequence GGGCGG²⁷. The structure of such control regions should resemble that shown here and in the other crystals mentioned.

We thank Dr A. Klug for suggesting the project, Drs B. Luisi

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1. Brown, D. D. *Harvey Lect.* **76**, 27–44 (1982).
2. Rhodes, D. & Klug, A. *Cell* **46**, 123–132 (1986).
3. Miller, J., McLachlan, A. D. & Klug, A. *EMBO J.* **4**, 1609–1614 (1985).
4. Rosenberg, U. B. *et al. Nature* **319**, 336–339 (1986).
5. Vincent, A., Colot, H. V. & Rosbach, M. *J. molec. Biol.* **186**, 149–166 (1985).
6. Hartshorne, T. A., Blumberg, H. & Young, E. T. *Nature* **320**, 283–287 (1986).
7. Rhodes, D. *EMBO J.* **4**, 3473–3482 (1985).
8. Sakonju, S. & Brown, D. D. *Cell* **31**, 395–405 (1982).
9. Fairall, L., Rhodes, D. & Klug, A. *Cell* (submitted).
10. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. *Cell* **19**, 13–25 (1980).
11. Bodenhausen, D. F., Sakonju, S. & Brown, D. D. *Cell* **19**, 27–35 (1980).
12. Gait, M. J., Matthes, H. W. D., Singh, M., Sproat, B. S. & Titmus, R. C. *Nucleic Acids Res.* **10**, 6243–6254 (1982).
13. Conner, B. N., Yoon, C., Dickerson, J. L. & Dickerson, R. E. *J. molec. Biol.* **174**, 663–695 (1984).
14. Wang, A. H.-J., Fujii, S., van Boom, J. H. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3968–3972 (1982).
15. McCall, M. J., Brown, T. & Kennard, O. *J. molec. Biol.* **183**, 385–396 (1985).
16. Sussman, J. L., Holbrook, S. R., Church, G. M. & Kim, S. H. *Acta crystallogr. A* **33**, 800–804 (1977).
17. Hendrickson, W. A. & Konnert, J. H. in *Biomolecular Structure, Conformation, Function and Evolution* Vol. 1 (ed. Srinivasan, R.) 43–57 (Pergamon, Oxford, 1981).
18. Wilson, A. J. C. *Nature* **150**, 152 (1942).
19. Arnott, S. & Hukins, D. W. L. *Biochem. biophys. Res. Commun.* **47**, 1504–1509 (1972).
20. Calladine, C. R. & Drew, H. R. *J. molec. Biol.* **178**, 773–782 (1984).
21. Arnott, S., Hukins, D. W. L. & Dover, S. D. *Biochem. biophys. Res. Commun.* **48**, 1392–1399 (1972).
22. Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W. & Hodgson, A. R. *J. molec. Biol.* **81**, 107–122 (1973).
23. Nilsson, L., Clore, G. M., Gronenborn, A. M., Brünger, A. T. & Karplus, M. *J. molec. Biol.* **188**, 455–475 (1986).
24. Drew, H. R. & Travers, A. A. *Cell* **37**, 491–502 (1984).
25. Haran, T. thesis, Weizmann Inst. (1986).
26. Shakked, Z. *et al. J. molec. Biol.* **166**, 183–201 (1983).
27. Kadonaga, J. T., Jones, K. A. & Tjian, R. *Trends biochem. Sci.* **11**, 20–23 (1986).
28. Rabinovich, D. & Shakked, Z. *Acta crystallogr. A* **40**, 195–200 (1984).
29. Watson, J. D. & Crick, F. H. C. *Nature* **171**, 737–738 (1953).
30. Franklin, R. E. & Gosling, R. G. *Acta crystallogr.* **6**, 673–677 (1953).
31. Langridge, R. *et al. J. molec. Biol.* **2**, 38–64 (1960).
32. Fuller, W., Wilkins, M. H. F., Wilson, H. R. & Hamilton, L. D. *J. molec. Biol.* **12**, 60–80 (1965).
33. Arnott, S. *Nature* **320**, 313 (1986).
34. Huber, P. W. & Wool, I. G. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1593–1597 (1986).

MATTERS ARISING

The mechanism of activation of porcine pepsinogen

IN a recent paper we studied the early events in the activation of porcine pepsinogen using fluorescence-detected stopped flow kinetics¹. We characterized two first-order decays when working with both native pepsinogen and a covalent conjugate between the fluorescent moiety 6-(*p*-toluidinyl)-naphthalene-2-sulphonyl- and pepsinogen¹. This result was demonstrated to be consistent with a model in which concurrent first-order transformations are undergone by two species of zymogen which are related by an ionization-dependent equilibration between them. (A sequential model was ruled out by an analysis of the data.) We hypothesized that the two species initiate two concurrent pathways of activation. Subsequently, James and Sielecki, in an extrapolation from their new crystallographic structure of porcine pepsinogen, also proposed two alternative pathways for activation². They did not explicitly suggest, however, that the two paths are followed simultaneously under a given set of conditions. It will be noted that their proposal bears a close resemblance to the model we had already worked out¹ based on the kinetic analysis described above. Most recently we have demonstrated that the pH-dependent segregation of pepsinogen molecules between alternative pathways persists at least through the process which exposes the active site³. This was shown by following the kinetics of binding of a fluorescent analogue of pepstatin to pepsinogen upon acidification. It was again observed that there are two first-order processes consistent with the concurrent reaction model described above.

The crystal structure of pepsinogen reported by James and Sielecki² permits a much clearer understanding of the origins of the changes in intrinsic fluorescence that we obtained¹. They noted that the

environment of at least four tyrosyl residues must be perturbed upon release of Lys 36P (residues 1P–44P comprise the activation peptide) from its electrostatic interaction with the carboxylates of the catalytically-active residues Asp 32 and Asp 215. The fluorescence changes that we observed occur on the millisecond time scale, and are followed by no further change for several seconds¹. This strongly suggests that very soon after acidification the activation peptide is released from its initial position in which it blocks the catalytic site².

The distinguishing conformational features of the two molecular species of acidified pepsinogen, which form a Bronsted acid-base conjugate pair, remain to be identified. Further studies by us, as well as results obtained by crystallographic analysis, may provide a means to this end.

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1. Auer, H. E. & Glick, D. M. *Biochemistry* **23**, 2735–2739 (1984).
2. James, M. N. G. & Sielecki, A. R. *Nature* **319**, 33–38 (1986).
3. Glick, D. M., Auer, H. E., Rich, D. H., Kawai, M. & Kamath, A. *Biochemistry* **25**, 1858–1864 (1986).

JAMES AND SIELECKI REPLY—Acid activation of porcine pepsinogen has long been known to involve conformational changes^{1,2} that can be reversed by rapidly returning the pH of the solution to neutrality. Conformational changes on a time scale of 5 ms–2 s have also been detected by stopped flow fluorescence kinetics.³ Analysis of these data suggests that there are two concurrent activation pathways for pepsinogen. Exposure of the active site of the enzyme occurs on roughly the same time scale (1–2 s). The kinetic

data for the binding of a fluorescent pepstatin analogue are also interpretable in a two concurrent pathway model.⁴

The crystal structure of porcine pepsinogen⁵ has shown that the pepsin active site is fully formed in the zymogen but that it is blocked competitively by the specific folding of the activation peptide (Leu1P–Leu44P). This blocking takes place in a manner different from that expected for productive substrate binding. The two alternative steps in the proposed activation pathway⁵ are concerned only with repositioning the activation peptide so that the scissile bond (Leu16P–Ile17P) can approach the catalytic aspartates. This cleavage step has a relatively longer half-time of ~28 s (ref. 6). Unfortunately, a static crystal structure cannot provide definitive data on dynamic events that are occurring at millisecond or second time scales. However, the three-dimensional structure allows us to propose the two alternative pathways for productive binding of the scissile peptide bond of pepsinogen in the unimolecular cleavage reaction. Whether our alternative binding modes are associated with relatively rapid fluorescence changes and the concurrent pathways so deduced remains to be seen.

We thank Drs Auer and Glick for the opportunity to read their manuscript⁴ prior to publication.

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1. Perlmann, G. E. *J. molec. Biol.* **6**, 452–463 (1963).
2. McPhie, P. *J. biol. Chem.* **247**, 4277–4281 (1972).
3. Auer, H. E. & Glick, D. M. *Biochemistry* **23**, 2735–2739 (1984).
4. Glick, D. M., Auer, H. E., Rich, D. H., Kawai, M. & Kamath, A. *Biochemistry* **25**, 1858–1864 (1986).
5. James, M. N. G. & Sielecki, A. R. *Nature* **319**, 33–38 (1986).
6. Al-Janabi, J., Hartsuck, J. A. & Tang, J. *J. biol. Chem.* **247**, 4628–4632 (1972).

The laboratory networking dilemma

from W.H. Jennings

Computer networking in the research laboratory is still a rarity. But growing demands for shared resources may prompt laboratories to make the connection . . . the question is when and how.

WITH the advent of microcomputers, data acquisition and control became dominated by systems dedicated to or embedded within the research instrument. The constraints of real-time operation and the limited computational capacity of these systems favours the transfer of raw data files to another machine for computationally intensive processing. While most scientists are familiar with this sort of transfer, local area networks that make the various computers within a department accessible to all department staff are still uncommon. Yet many research laboratories could benefit enormously from systems that distribute data to the computer most appropriate for a particular computational task.

Presently, moving data between machines is often accomplished by media transfer using magnetic tape or some form of disk. The difficulties of media transfer have encouraged the development of file transfer techniques that use serial transmission to transport data, often via telephone lines. Although vendors have been dragging their feet in devising common file format and transfer protocols for larger machines, several reasonably documented protocols exist for personal computers, and some of these have been adapted to large machines. There is then the potential for any computer-based laboratory instrument to transfer data to a departmental host machine or even some remote computer.

But realizing this potential at a level truly useful to laboratory scientists requires networking technology, and this technology is in flux. Herein lies the first element of the dilemma: when is it appropriate for a laboratory to implement a computer network?

Choosing the moment

Unfortunately, very few laboratories have the luxury of implementing a network *de novo* because of the computer-based instruments and/or the relatively expensive departmental computers they may have already accumulated. Interconnecting a variety of vendors' products, some of which may be obsolete, can pose problems. And of the many network products now available, there is probably no one product that is appropriate for all laboratory applications.

Nonetheless, rather straightforward means are available to remedy most of these complications, even though in some cases the solutions may be only marginally

adequate. Networks are intrinsically expandable and extensible and their future value may be far more important than present economic or productivity gains. Current network standards offer both physical connectivity with fairly long lifetimes and protocols that, being software,

cannot be upgraded with a network interface can gain network access through terminal concentrators, which accept RS-232 serial transmissions and perform the necessary hardware and software functions to interface to the network. This access is limited in functionality and

Computer networks at work

Du Pont Experimental Station
Wilmington, Delaware

An Ethernet system
connects 40 buildings over
150 acres

Upjohn pharmaceutical R&D complex
Kalamazoo, Michigan

A network comprised of
5 km of cable is tied to
seven systems in separate
facilities

General Motors research facility
Warren, Michigan

Over 300 IBM and DEC
microcomputers are linked
by a minicomputer inter-
mediary

can be upgraded. There is then no compelling argument here to wait for further developments.

In fact, the decision to implement a laboratory network does not depend so much on issues of technology as on the commitment of laboratory personnel and funding. Even though its tangible portions may be limited to cables and connectors, a network requires considerable management and support, and in most labs these functions must be provided by existing personnel. Because the majority of networks are implemented at the departmental level rather than in top administrative or corporate echelons, the laboratories that will share the network usually have to dip into their own financial and personnel resources to sustain it.

The department that has decided to commit itself to networking confronts the second element of the dilemma: how can a laboratory network its computer resources? A variety of consultants and manufacturers provide technology support for networking projects, but the development of in-house expertise is desirable for both implementation and subsequent operations management.

Casting the net

The volume and characteristics of the data handled within a laboratory will determine a rough lower limit for network capacity. Generous margins should be added to allow expansion. Where very high data volume is required, a limited local network with a gateway to other networks can be a worthwhile option.

"Dumb" terminals and instruments that

speed; however, it is adequate for the majority of these devices.

The nearly ubiquitous personal computer can be connected either directly through an interface card or indirectly through a terminal concentrator. Terminal emulation software disguises the personal computer so that it appears to a remote computer as one of a variety of popular terminals. The networked PC can thus perform interactive file manipulation and execute programs on the remote system. Most emulation software provides for file transfer to or from the remote as well.

Techniques for access and sharing of files and programs throughout a network are being extensively tested on advanced networks of more than 1,000 nodes. Meanwhile the next generation of computer systems, the personal workstation, is beginning to appear in the research laboratory. This device has a large memory, high resolution graphics and computational power exceeding that of some minicomputers. Most are sold with a network interface and AT&T's operating system UNIX, which already contains many networking functions.

The goal of networking is to make efficient use of computer resources, providing access to any level of computational power that the user might require without the bother of juggling cables, connections or conversions. For any laboratory ready to commit modest resources, this goal is already in sight. □

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The binary breakthrough

From data acquisition to manuscript submission, computers aid research every step of the way and the computers often jump in where their owners may fear to tread.

WITHIN weeks, California-based IntelliGenetics will be releasing molecular biology software that the company says can perform over 50 **analyses of peptide and nucleic acid structure**, both primary and secondary (Reader Service No. 100). The

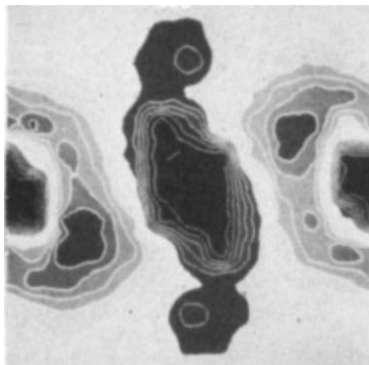


PC/GENE is based on published methods.

PC/GENE microcomputer package will search for specified sequences, predict helix distortion and hairpins, find probable coding regions and simulate restriction digestions. Capable of drawing over 20 different types of plots, PC/GENE runs on IBM PCs and compatibles with graphic cards. IntelliGenetics' commercial price is \$3,500 (US); academic institutions can purchase the package for \$2,500 (US) and BIONET subscribers gain access to PC/GENE at no additional cost. Programs allow file transmission between PC/GENE and BIONET, the company's mainframe timesharing system.

Two software packages for **protein analysis and restriction mapping** are among DNASTAR's offerings for molecular biologists (Reader Service No. 101). The company says its protein programs calculate plots of secondary structure and hydrophilicity by two different methods, and can determine optimal DNA probes, titration curves, isoelectric points and molecular weights. DNASTAR asks about \$3,000 (US) for that package. The \$990 (US) R-MAP program derives circular or linear restriction maps from enzyme digestion data by way of an algorithm that the company claims is unique. Both products run on IBM PCs and compatibles.

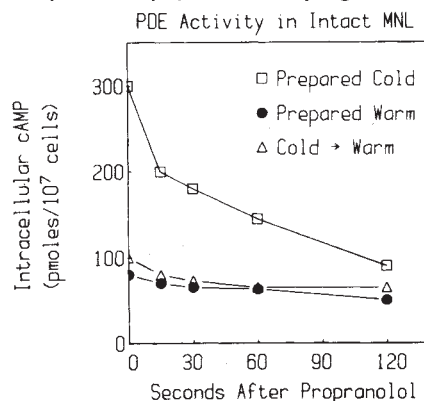
Chemical Design Ltd thinks its latest addition to the Chem-X molecular modelling family will make traditional laboratory notebooks obsolete (Reader Service No. 102). Chem DBS-1 is a **database software module** that also automatically records the results of Chem-X calculations and accompanying comments. Energy



A Chem-X energy analysis map for histamine. values, significantly positive or negative map volumes and user-defined geometries can be stored in DBS-1, in addition to structural information on several hundred molecules. "Pages" containing records of Chem-X calculations such as molecular fitting and spatial analyses can be located later by page number, date of entry, type of calculation and molecular name. Chemical Design's turnkey system MicroGRAF-4, which starts at \$95,000 (US), incorporates Chem-X software for DEC MicroVAX II. DBS-1 will be demonstrated at September's ACS meeting in Anaheim.

Graphic detail

Plotting **presentable graphs** is the aim of software from Academic Press called Graph-PAD (Reader Service No. 103). Using an IBM PC with graphics capabilities and a Hewlett-Packard plotter or compatible equipment, the program can



Academic Press's program can graph multiple experiments.

plot bars, data points or curves from any of 10 available equations. Graph-PAD accepts data typed into a spreadsheet or contained in external files. The program can also digitize existing graphs via a plotter pen. Data manipulation features include calculations of average replicate values,

error bars, linear regressions and logarithmic transformations. Academic Press says no computer experience is necessary to be able to create graphs in less than 10 minutes and offers a \$5 (US) demonstration disk to make its point. The complete diskette and its 98-page manual cost \$340 (US).

Janssen's life sciences division now offers a **novel interpretation of numerical tabular data** in the form of SpectraMap (Reader Service No. 104). Unlike bar, line or pie chart graphs, Janssen's program elucidates the ratios generated from problems involving many parameters. SpectraMap uses factor analysis and advanced computer graphics to arrive at a display complex enough to require instructions for interpretation. The company says its profiles can be applicable to studies in disciplines ranging from biophysics to epidemiology. Janssen provides the service on a piecemeal basis starting at £150 (UK) per graph. Alternatively, licensing of SpectraMap's PC version for £2,535 (UK) can be arranged for regular users.

Mathematical equations are unruly entities in the word-processing world. But recently a Massachusetts company, Technical Support Software, began marketing

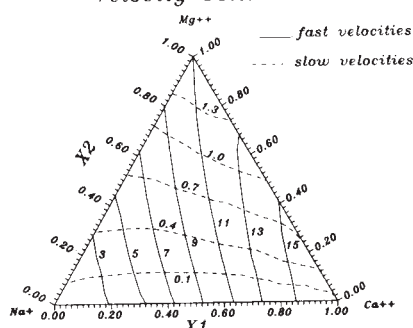


EXACT puts mathematics in the picture.

typesetting software that enables scientific and mathematical expressions to be incorporated into standard word-processing text and printed on garden-variety printers (Reader Service No. 105). Called EXACT, the program loads into RAM before the word processor and performs split-screen editing within the context of the target document. The editing mode displays characters exactly as they will appear in the printed document, automatically re-scaling radicals, braces, brackets, parentheses and boxes. EXACT's vocabulary includes 20 different fonts and over 1,000 symbols and characters. TSS guarantees its program on any word processor and sells a demo disk at \$5 (US); the real thing cost \$475 (US).

The chore of **visualizing relationships amongst large data volumes** is simplified with GRAPHIER. Golden Software Inc's answer to plotting (Reader Service No. 106). With five types of regression curves, four types of error bars and graphs includ-

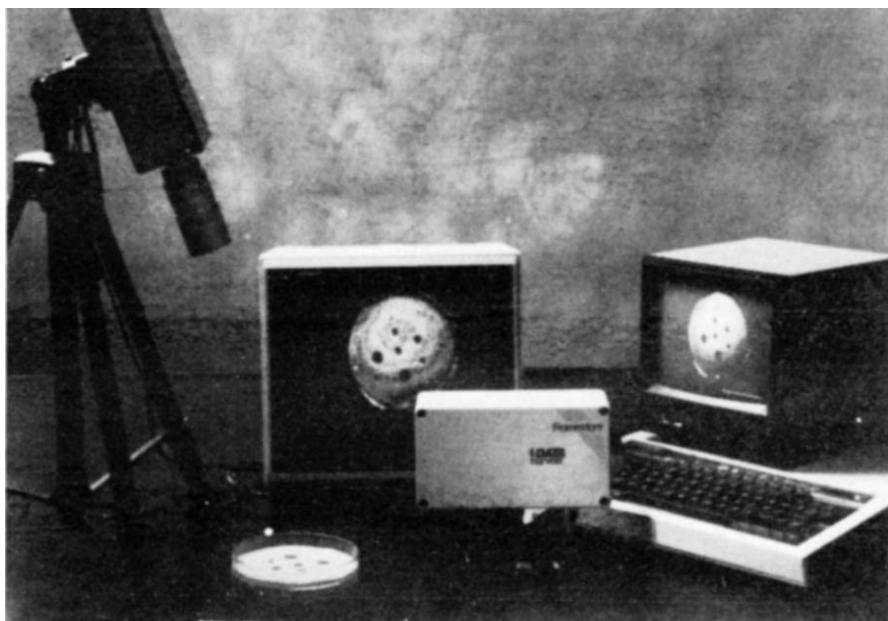
ing linear-linear, linear-log and triangular. GRAPHER can unveil the implications obscured by sheer numbers. It will



Complex relationships come to light with GRAPHER.

generate grid lines and any number of data scales or data lines can be combined on one graph. The \$199 (US) program's worksheet loads from keyboard or external data files; it will annotate graphs with text. Golden says GRAPHER runs on IBM PCs and compatibles with several kinds of graphics adaptors. A \$10 (US) demonstration disk can be supplied upon request.

A personal software series for chemists comes from ECS Chemical Systems in the UK (Reader Service No. 107). From CHEMTALK, a £1,000 (UK) program, to CHEMBASE, the series' modelling component. ECS's software aids in the storage, retrieval and display of chemical information. A hand-held mouse can select commands, draw structures or assemble reactions. The graphics-based word processor CHEMTEXT accepts transfers from CHEMBASE, while CHEMHOST and CHEMTALK link mainframe databases to the PC software. ECS expects its system to accelerate research formerly harnessed with the tedium of producing polished reports by hand. The IBM-compatible software boasts 500 different help screens for the first-time user.



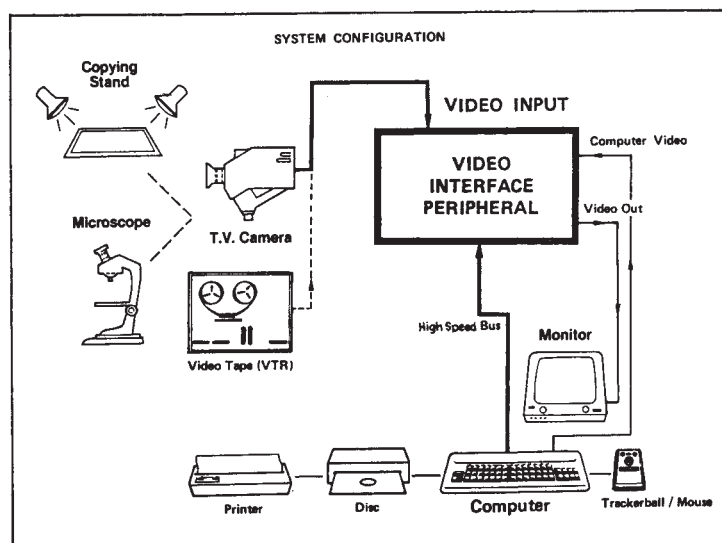
In this FrameStore configuration, the right monitor shows a monochrome digitized image and the left displays the image with false colour generated by Data Harvest software.

Worth 1,000 words?

A menu-driven image analysis system from Sight Systems uses two video frame stores with 512×512 resolution for image storage (Reader Service No. 108). The grey and binary framestores of the company's Video Interface Peripheral (VIP) work in tandem at video rate; the menu that occupies one quarter of the screen provides a space for text and headings for routines or measuring processes. Sight Systems' analyser also achieves fast thresholding, according to the company, providing an image that is simpler to understand than unembellished images. The computer can read or write to any pixel in the binary store, grey store or menu area at any time. Because the VIP can analyse images live with no adverse affects, pseudo-real time analysis is possible, says Sight Systems. The company sells VIP in configurations costing between £2,000 and £4,500 (UK).

Data Harvest has pulled the price of its FrameStore analyser down to £395 (UK) (Reader Service No. 109). The company says its economy-line system can distinguish up to 64 discrete levels of image intensity and provide resolution of 192×256 pixels. A microcomputer allows manipulation, processing or transfer of digitized data. Because outlines can be identified through contrast enhancement, FrameStore can make area measurements/ratios calculations by pixel count. The analyser accepts video signals from sources such as video cameras, cassette recorders and disks and can be purchased separately or as part of a complete video system.

A desktop X-ray microanalysis system has been introduced as an "economic



Slight Systems has designed its image analyser to accept many different video sources and give standard video output. The Video Interface Peripheral can also be used as a video event recorder. The system employs a high order of noise reduction, and a phase lock circuit synchronizes the VIP to the incoming video.



Link Systems backs up the QX200 with full support services. alternative" by Link Systems in High Wycombe (Reader Service No. 110). The company says its model QX200 offers display capabilities ranging from on-screen

peak seek to labelling and KLM identification, and stores data via 3.5-inch floppy drives. Extensive data acquisition and manipulation capabilities are part of the QX200 package, as well as spectrum processing. A mouse is optional to simplify operating procedures, and the £24,000 (UK) system includes a bidirectional printer/plotter. Link says that in addition to the quantitative X-ray microanalysis program included with the package, an extended range of application software is available.

Designs for data

Computer analysis without computer expertise is the promise of Macmillan software's ASYSTANT package (Reader Service No. 111). The new software, says Macmillan, puts advanced application functions in a menu-driven format for ease of use. The company's basic \$495 (US) package performs **integrated data reduction and analysis** with high-resolution colour graphics: an \$895 (US) version

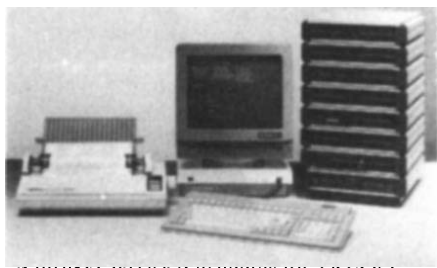
called ASYSTANT+ adds data acquisition capabilities to the basic software. Macmillan's programs are designed for IBM personal computers.

A PC program from Laboratory Technologies Corp builds a bridge between the company's LABTECH NOTEBOOK data acquisition software and standard PC/MS-DOS applications programs (Reader Service No. 112). LABTECH Real Time Access, a \$195 (US) option for the \$895 (US) acquisition package, moves data samples from NOTEBOOK directly into spreadsheet, statistical data analysis, data base management and display programs. Hence the numbers generated by an external process and acquired by NOTEBOOK could appear in real time, continuously updated, on a Lotus 1-2-3 worksheet.

Nearly a decade of experience in chemical database management has given rise to MACCS-II, an integrated and **customizable information system** from Molecular Design (Reader Service No. 113). The company claims that the advantage of its latest system lies in its potential to be

configuration that automatically maintains mirror images of active sample files on a separate disk.

In designing its LIMS, Trivector Systems tried to develop a network micro-computer approach rather than build the



system around a centralized super-mini-computer (Reader Service No. 115). The result: TRINET, an **information manager** that Trivector hopes will provide both modest beginnings and considerable potential for expansion. At the heart of the system, a dedicated information manager with ports for up to 16 stations coordinates data management, whilst the stations are engaged in manual input, enquiries, program development and data transfer or collection. A database management package with its own screen and report generation programs makes LIMS design quick and easy, according to Trivector, which asks £15,000 (UK) and up for TRINET.

Guides to programs

A brochure detailing Fisher's **toxic substance database** shows full-colour examples of the program's display (Reader Service No. 116). These glimpses of ChemPro give a fair idea of the software's functioning, which gives information on any of 4,100 hazardous chemicals most frequently found in research laboratories, according to the company. Fisher kept the program's format uncomplicated so that substances could be located quickly in an emergency, and there are special keys for fires, spills and poisonings. ChemPro runs on an IBM AT.

The **scientific software and educational programmes** marketed by Autoscribe Ltd are reviewed in the company's latest catalogue (Reader Service No. 117). Autoscribe offers software such as Molecular Presentation Graphics for chemical structure drawing and Datalyst for chemical databases. Educational programmes are available on various media. The company hopes to expand both its education and software ranges shortly. □

These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.

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Reader Service No.50

Life for LIMS

Tips on improving the performance and security of laboratory information management systems (LIMS) abound in a **technical bulletin** from Beckman (Reader Service No. 114). The bulletin uses Beckman's recent revision of its own CALS LabManager product to illustrate measures that can simplify organization and protect data copies. By dividing active samples from completed samples within the database, Beckman reports, the company was able to improve search time for many retrievals and create back-up versions of the completed base. Such insurance against disk drive failures provides the rationale for Beckman's LabManager Safeguard as well, an optional

Astronomy

Astrophotography for the Amateur. By MICHAEL COVINGTON. Cambridge University Press:1985. Pp.168. Hbk ISBN 0-521-25391-8. £15, \$24.95.

Astrophysics of Active Galaxies and Quasi-Stellar Objects. By J.S. MILLER. Oxford University Press:1985. Pp.519. Hbk ISBN 0-19-8557-17-5. £37.50.

Beyond the Black Hole: Stephen Hawking's Universe. By JOHN BOSLOUGH. Fontana/Collins:1986. Pp.127. Pbk ISBN 0-00-636652-X. £2.50.

Birth and Evolution of Massive Stars and Stellar Groups. W. BOLAND and H. VAN WOERDEN (eds). D. Reidel:1985. Pp.377. Hbk ISBN 90-277-2135-1. Dfl. 150, £41.75, \$59.

Early History of Cosmic Ray Studies: Personal Reminiscences with Old Photographs. YATARO SEKIDO and HARRY ELLIOT (eds). D. Reidel:1985. Pp.444. Hbk ISBN 90-277-2083-5. Dfl. 250, £69.25, \$89.

Mass Loss from Red Giants. MARK MORRIS and BEN ZUCKERMAN (eds). Reidel:1985. Pp.320. ISBN 90-277-2075-4. Dfl. 135, \$49.

The Planet Sun (enlarged 1985 edition). By TASSOS A. KAROUSATOS. Laboratory of Magneto-Optical Research, 43 Levidou Street, GR-145 63. Kifissia, Athens, Greece:1985. Pbk np.

Supernovae. By PAUL and LESLEY MURDIN. Cambridge University Press:1985. Pp.185. Hbk ISBN 0-521-30038-X. £12.95, \$24.95.

Physics

Advanced Fracture Mechanics. By MELVIN F. KANNINEN and CARL H. POPELAR. Oxford University Press:1985. Pp.563. ISBN 0-19-503532-1. £40.

Amorphous Solids and the Liquid State. NORMAN H. MARCH, ROBERT A. STREET and MARIO TOSI (eds). Plenum:1985. Pp.539. Hbk ISBN 0-306-41947-5. Np.

Cryogenic Systems, 2nd Edn. By RANDALL F. BARRON. Oxford University Press:1985. Pp.507. ISBN 0-19-503567-4. £60.

Desorption Induced by Electronic Transitions. W. BREINIG and D. MANZEL (eds). Springer-Verlag:1985. Pp.291. ISBN 3-540-15593-7/0-387-15593-7. DM 92.

Dynamical Phenomena at Surfaces, Interfaces and Superlattices. F. NIZZOLI, K.H. RIEDER and R.F. WILLIS (eds). Springer-Verlag:1985. Pp.329. ISBN 3-540-15505-8/0-387-15505-8. DM 89.

Elements of Modern Optical Design. By DONALD C. O'SHEA. Wiley:1985. Pp.402. Hbk ISBN 0-471-07796-8. £40.85.

Foundations of Quantum Mechanics II. By G. LUDWIG. Springer-Verlag:1985. Pp.416. Hbk ISBN 3-540-13009-8. DM 228.

Fundamental Interactions in Low-Energy Systems. P. DALPIAZ, G. FIORENTINI and G. TORELLI (eds). Plenum:1985. Pp.501. ISBN 0-306-42002-3. Np.

Fourier Techniques and Applications. JOHN F. PRICE (ed.). Plenum:1985. Pp.231. Hbk ISBN 0-306-42100-3. Np.

Introduction to Concepts and Theories in Physical Science (2nd Edn.). By GERALD HOLTON, revised and with new material by STEPHEN G. BRUSH. Princeton University Press:1985. Pbk ISBN 0-691-08384-3. \$19.95.

Molecular Electromagnetism. By A. HINCHLIFFE and R.W. MUNN. Wiley:1985. Pp.252. Pbk ISBN 0-471-90721-9. Pbk £9.95.

Applied Biological Sciences

Advances in Cancer Research, Vol.45. Academic:1985. Pp.324. Hbk ISBN 0-12-006645-9. £48, \$55.

Advances in Human Genetics, Vol.15. H. HARRIS and K. HIRSCHHORN (eds). Plenum:1986. Pp.299. Hbk ISBN 0-306-42155-0. Np.

Advances in Veterinary Science and Comparative Medicine, Vol.30. CHARLES E. CORNELIUS and CHARLES F. SIMPSON (eds). Academic:1985. Pp.339. Hbk ISBN 0-12-039299-1. £52, \$59.50.

Advances in Pain Research and Therapy, Vol.9, Proceedings of the Fourth World Congress on Pain. HOWARD L. FIELDS, RONALD DUBNER and FERNANDO CERVERO (eds). Raven Press:1985. Pp.951. Hbk ISBN 0-88167-121-5. \$153.

Animal Behaviour and Its Applications. By DEREK V. ELLIS. Lewis Publishers:1985. Pp.329. Hbk ISBN 0-87371-020-7. £27.55.

Aortitis: Clinical, Pathological and Radiographic Aspects. ADAM LANDE, YAHYA M. BERKMAN and HUGH A. McALLISTER, Jr (eds). Raven:1985. Pp.288. Hbk ISBN 0-88167-141-X. \$63.50.

Atlas of Cancer in Scotland 1975-1980: Incidence and Epidemiological Perspective. I. KEMP, P. BOYLE, M. SMANS and C. MUIR (eds). IARC Scientific Publications, Lyons:1985. Pp.282. Hbk ISBN 928-321172-3. £35.

The Autoimmune Disease. NOEL R. ROSE and IAN R. MACKAY (eds). Academic:1986. Pp.727. Hbk ISBN 0-12-596920-1. £75, \$85.

Bacteria and Complement. Current Topics in Microbiology and Immunology, Vol.121. MICHAEL LOOS (ed.). Springer-Verlag:1985. Pp.191. Hbk ISBN 3-540-15877-4. Np.

Biological Effects and Dosimetry of Static and ELF Electromagnetic Fields. M. GRANDOLFO, S.M. MICHAELSON and A. RINDI (eds). Plenum:1985. Pp.697. Hbk ISBN 0-306-41923-8. Np.

Biology of Sewage Treatment and Water Pollution Control. By K. MUDRACK and S. KUNST. Ellis Horwood:1986. Pp.193. Hbk ISBN 0-85312-912-6. £27.50.

Biorhythms and Epilepsy. A. MARTINS DA SILVA, C.D. BINNIE and H. MEINARDI (eds). Raven:1985. Pp.254. Hbk ISBN 0-88167-124-X. \$46.50.

Capnography in the Operating Room, an Introductory Directory. By W. SAMUEL MAY Jr, JAMES E. HEAVNER, DOROTHY McWHORTER and GABOR RACZ. Raven Press:1985. Pp.64. Pbk ISBN 0-88167-128-2. \$14.00.

Cell Culture and Somatic Cell Genetics of Plants. Vol.2, Cell Growth, Nutrition, Cytodifferentiation and Cryopreservation. INDRA K. VASIL (ed.). Academic:1985. Pp.330. Hbk ISBN 0-12-715002-1. £42.50, \$49.50.

Cell Membranes and Cancer. T. GALEOTTI, A. CITTADINI, G. NERI, S. PAPA and L.A. SMETS (eds). Elsevier:1985. Pp.446. Hbk ISBN 0-444-80723-3. Dfl 230, \$85.25.

Chemotherapy of Parasitic Diseases. WILLIAM C. CAMPBELL and ROBERT S. SHAW (eds). Plenum:1986. Pp.655. Hbk ISBN 0-306-42029-5. Np.

Congenital Adrenal Hyperplasia. MARIA I. NEW (ed.). New York Academy of Sciences:1985. Pp.290. ISBN 0-89766-311-X. Np.

Contact Allergy Predictive Tests in Guinea Pigs. Current Problems in Dermatology, Vol.14. K.E. ANDERSEN and H.I. MAIBACH (eds). Karger:1985. Pp.300. Hbk ISBN 3-8055-4053-1. Sfr. 162, DM 194, £50.70, \$69.00.

Contemporary Issues in Pesticide Toxicology and Pharmacology. By JUDITH K. MARQUIS. Karger:1986. Pp.108. Hbk ISBN 3-8055-4215-1. Sfr. 89, DM 107, £27.90, \$38.

Control of Animal Cell Proliferation, Vol.1. ALTON L. BOYNTON and HYAM L. LEFFERT (eds). Academic:1985. Pp.557. Hbk ISBN 0-12-123061-9. £60.50, \$68.50.

Control of Pig Reproduction II. Proceedings of the Second International Conference on Pig Production Held at Columbia Missouri, USA. G.R. FOXCROFT, D.J.A. COLE and BARBARA J. WEIR (eds). Journal of Reproduction and Fertility:1985. Pp.266. Hbk ISBN 0-906545-10-2. \$63.

Deep Dyslexia. MAX COLTHEART *et al.* (eds). Routledge & Kegan Paul:1986. Pp.444. Pbk ISBN 0-7102-0834-0. Pbk £8.95.

Dietary Fiber and Obesity. Current Topics in Nutrition and Disease, Vol.14. Alan R. Liss:1985. Pp.126. Hbk ISBN 0-8451-1613-4. £17.

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Evaluation of Cyclamate for Carcinogenicity. Commission on Life Sciences, National Research Council. National Academy Press, Washington, US:1985. Pp.196. Pbk ISBN 0-309-03546-5. Pbk £14.50.

Forest Ecosystems: Concepts and Management. By RICHARD H. WARING and WILLIAM H. SCHLESINGER. Academic:1985. Pp.340. Hbk ISBN 0-12-735440-9. £39.50, \$45.

Gaba and Mood Disorders: Experimental and Clinical Research. L.E.R.S Monograph Series, Vol.4. G. BARTHOLOMI, K.G. LLOYD and P.L. MORSELLI. Raven Press:1986. Pp.240. Hbk ISBN 0-88167-129-0. \$32.50.

Gene Manipulations in Fungi. J.W. BENNETT and LINDA L. LASURE (eds). Academic:1986. Pp.558. Hbk ISBN 0-12-088640-5. £65, \$75.

Genetic Approaches to Microbial Pathogenicity. Current Topics in Microbiology and Immunology, Vol.118. W. GOEBEL (ed.). Springer-Verlag:1985. Pp.283. Hbk ISBN 3-540-15597-X. DM 160.

Genetic Basis of Biochemical Mechanisms of Plant Disease. JAMES V. GROTH and WILLIAM R. BUSHNELL (eds). The American Phytopathological Society Press:1985. Pp.157. Pbk ISBN 0-89054-068-3. \$24.

Genetic Perspectives in Biology and Medicine. EDWARD D. GARBER (ed.). The University of Chicago Press:1986. Pp.500. Hbk ISBN 0-226-28721-5; pbk ISBN 0-226-28216-3. Hbk £25.50; pbk £10.25.

Genetics, Cell Differentiation and Cancer. Bristol-Myers Cancer Symposia, Vol.7. PAUL A. MARKS (ed.). Academic:1985. Pp.209. Hbk ISBN 0-12-473060-4. £22.50, \$25.

Genetics and the Law III. AUBREY MILUNSKY and GEORGE J. ANNAS (eds). Plenum:1985. Pp.514. Hbk ISBN 0-306-41983-1. Np.

Genetics in Clinical Oncology. R.S.K. CHAGANTI and JAMES L. GERMAN (eds). Oxford University Press:1985. Pp.280. Hbk ISBN 0-19-503609-3. £35.

Hormonally Responsive Tumours. VINCENT P. HOLLANDER (ed.). Academic:1985. Pp.558. Hbk ISBN 0-12-352560-8. £59, \$68.

Humour Therapy in Cancer, Psychosomatic Diseases, Mental Disorders, Crime, Interpersonal and Sexual Relationships. By BRANKO BOKUN. Vita Books, 22 Chelsea Square, London SW3, UK:1986. Pp.221. Pbk ISBN 0-9510525-0-0. £5.00.

Hyperalgesia: A Guide for Clinicians. MITCHELL V. KAMINSKI Jr. (ed.) Dekker:1985. Pp.744. Hbk ISBN 0-8247-7375-6. \$95 (US and Canada), \$114 (other countries).

Implantation of the Human Embryo. R.G. EDWARDS, JEAN M. PURDY and P.C. STEP-TOE (eds). Academic:1985. Pp.459. Hbk ISBN 0-12-232455-2. £52, \$59.50.

Interrelationship Among Aging, Cancer and Differentiation. The Jerusalem Symposia on Quantum Chemistry and Biochemistry, Vol.18. BERNARD PULLMAN, PAUL O.P. T'SO and EDMUND L. SCHNEIDER (eds). D. Reidel:1985. Pp.345. Hbk ISBN 90-277-2117-3. Dfl 145, £40.25, \$57.

Large-Scale Mammalian Cell Culture. JOSEPH FEDER and WILLIAM R. TOLBERT (eds). Academic:1985. Pp.159. Hbk ISBN 0-12-250430-5. £22.50, \$25.

The Limbic System: Functional Organization and Clinical Disorders. BENJAMIN K. DOANE and KENNETH F. LIVINGSTON (eds). Raven Press:1985. Pp.365. Hbk ISBN 0-88167-134-7. \$64.50.

Mathematical Physics. By ROBERT GEROCH. The University of Chicago Press:1986. Pp.351. Hbk ISBN 0-226-28861-7. £25.50.

Mechanism of Menstrual Bleeding. D.T. BAIRD and E.A. MICHIE (eds). Raven:1985. Pp.276. Hbk ISBN 0-88167-094-4. \$43.50.

Medicinal Chemistry Research in India. By HAR-KISHAN SINGH, A.S. CHAWLA and V.K. KAPOOR. *National Information Centre for Drugs and Pharmaceuticals, Lucknow, India:1985. Pp.184. ISBN 81-85042-00-4. \$35.*

Medicinal Plants in Tropical West Africa. By B.E.P. OLIVER-BEVER. *Cambridge University Press: 1986. Pp.375. Hbk ISBN 0-521-26815-X. £40, \$75.*

Methods for Nutritional Assessment of Fats. JOYCE BEARE-ROGERS (ed.). *American Oil Chemists Society:1985. Pp.255. Hbk ISBN 0-935315-11-X. \$50.*

Methods in Porphyrin Photosensitization. *Advances in Experimental Medicine and Biology.* Vol.193. DAVID KESSEL (ed.). *Plenum:1985. Pp.352. Hbk ISBN 0-306-42210-7. Np.*

Migraine: Clinical and Research Advances. *Fifth International Migraine Symposium, London 1984.* F. CLIFFORD ROSE (ed.). *Karger:1985. Pp.280. Hbk ISBN 3-8055-4039-6. Sfr. 175, DM 210, £54.70, \$74.50.*

Migraine (new edn.). By OLIVER SACKS. *Duckworth:1986. Pp.270. Hbk ISBN 0-7156-2087-8. Np.*

Models for Biomedical Research: A New Perspective. *Commission on Life Sciences, National Research Council. National Academy Press:1985. Pp.180. Pbk ISBN 0-309-03538-4. £19.60.*

Molecular Biology and Biotechnology. J.M. WALKER and E.B. GINGOLD (eds). *Royal Society of Chemistry:1986. Pp.340. Pbk ISBN 0-85186-985-8. Pbk £25, \$45.*

Molecular Dynamics and Protein Structure. JAN HERMANS (ed.). *University of North Carolina Printing Dept.:1985. Pp.195. Pbk. \$10.*

Monoclonal Antibodies Against Bacteria, Vol.II. ALBERTO J.L. MACARIO and EVERLY CONWAY de MACARIO (eds). *Academic:1986. Pp.335. Hbk ISBN 0-12-463002-2. £42, \$49.*

New Directions for Biosciences Research in Agriculture: High Reward Opportunities. *National Academy Press:1985. Pp.122. Pbk ISBN 0-309-03542-2. £10.55.*

Non-Hodgkin's Lymphomas: New Techniques and Treatments. *Fourth Cancer Research Workshop, Grenoble 1984.* J.J. SOTTO, C. VROUSOS, M.F. SOTTO and F. VINCENT (eds). *Karger:1985. Pp.232. Hbk ISBN 3-8055-4064-7. Sfr 162, DM 194, £50.70, \$69.*

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Pharmacokinetics of Cardiovascular, Central Nervous System and Antimicrobial Drugs. *Royal Society of Chemistry, London:1985. Pp.462. Hbk ISBN 0-85186-937-8. £71, £39.*

The Pathologist and the Environment. DANTE G. SCARPELLI, JOHN E. CRAIGHEAD and NATHAN KAUFMAN (eds). *William & Wilkins: 1985. Pp.239. ISBN 0-683-07516-0. £47.50.*

Pathology of Unusual Malignant Cutaneous Tumours. MARK R. WICK (ed.). *Dekker:1985. Pp.440. Hbk ISBN 0-8247-7377-2. (USA & Canada) \$69.75, (other countries) \$83.50.*

Pedigree Analysis and Human Genetics. By ELIZABETH A. THOMPSON. *The John Hopkins University Press:1986. Pp.213. Hbk ISBN 0-8018-2790-6. £28.50.*

The Peptides: Analysis, Synthesis, Biology. Vol.7. *Conformation in Biology and Drug Design.* VICTOR J. HRUBY (ed.). *Academic:1985. Pp.495. Hbk ISBN 0-12-304207-0. £85.50 \$99.*

Pocket Atlas of Cranial Magnetic Resonance Imaging. By VICTOR M. HAUGHTON and DAVID L. DANIELS. *Raven:1986. Pp.61. Pbk ISBN 0-88167-171-1. Pbk \$14.*

Population Genetics in Forestry: Proceedings, Göttingham 1984. *Lecture Notes in Biomathematics.* 60. H.R. GREGORIUS (ed.). *Springer-Verlag:1985. Pp.287. Pbk ISBN 3-540-15980-0. DM 43.*

Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and

Heart. MICHAEL E. PHELPS *et al.* (eds). *Raven: 1985. Pp.690. ISBN 0-88167-118-5. \$98.50.*

Progress in Clinical Biochemistry and Medicine. Vol.2, Oncogenes and Human Cancer, Blood Groups in Cancer, Copper and Inflammation, Human Insulin. (Contributors by) T.L.J. BOEHM, U. DEUSCHLE, W.J. KUHNS, R. OBERMEIER, F.J. PRIMUS, U. WESER and M. ZOLTOBROCKI. *Springer-Verlag:1985. Pp.163. Hbk ISBN 3-540-15567-8. DM 84.*

Progress in Nucleic Acid Research and Molecular Biology, Vol.32. WALDO E. COHN and KIVIE MOLDAVE (eds). *Academic:1985. Pp.340. Hbk ISBN 0-12-540032-2. £43.50, \$49.50.*

Prostanoids: Pharmacological, Physiological and Clinical Relevance. By P.K. MOORE. *Cambridge University Press:1985. Pp.263. Hbk ISBN 0-521-26081-7; pbk ISBN 0-521-27827-9. Hbk £32.50, \$54.50; pbk £12.50, \$19.95.*

Protective Effect of BCG in Experimental Tuberculosis. By D.W. SMITH. *Karger:1985. Pp.97. ISBN 3-8055-4089-2. DM 107, \$38, £22.*

The Role of the Registry in Cancer Control. D.M. PARKIN, G. WAGNER and C.S. MUIR (eds). *International Agency for Research on Cancer:1985. Pp.155. ISBN 92-832-1166-9. £10.*

Serotonin and Microcirculation. Progress in Applied Microcirculation, Vol.10. R.S. RENEMAN and A. BOLLINGER (eds). *Karger:1986. Pp.92. Hbk ISBN 3-8055-4163-5. SwFr. 66, DM 79, £20.70, \$28.25.*

Survey of Drug Research in Immunologic Disease. Vol.6. Noncondensed Aromatic Derivatives. By V. ST. GEORGIEV. *Karger:1985. Pp.586. ISBN 3-8055-3962-2. DM 588, \$208.75.*

Synthetic Peptides as Antigens. CIBA FOUNDATION SYMPOSIUM 119. *Wiley:1986. Pp.307. ISBN 0-471-99838-9. £27.50.*

Testicular Cancer. Progress in Clinical and Biological Research, Vol.203. SAAD KHOURY, RENE KUSS, GERALD P. MURPHY, CHRISTIAN CHATELAIN and JAMES P. KARR (eds). *Alan R. Liss:1985. Pp.774. Hbk ISBN 0-8451-5053-7. £75.*

Textbook of Adverse Drug Reactions, 3rd Edn. D.M. DAVIES (ed.). *Oxford University Press:1986. Pp.814. Hbk ISBN 0-19-261479-7. £75.*

The Thorax, Part A. Lung Biology in Health and Disease. Vol.29. CHARIS ROUSSOS and PETER T. MACKLEM (eds). *Dekker:1985. Pp.1,616. Part A. Hbk ISBN 0-8247-7299-7. Part B. Hbk ISBN 0-8247-7300-4. £252 (sold only as a set).*

TNM-Atlas: Illustrated Guide to the TNM/pTNM-Classification of Malignant Tumour, 2nd Edn. *International Union Against Cancer. B. SPIESSL, P. HERMANEK, O. SCHEIBE and G. WAGNER (eds). Springer-Verlag:1985. Pp.269. Pbk ISBN 3-540-13443-3. DM 30.*

Transformation Assay of Established Cell Lines: Mechanisms and Application. *Proceedings of a Workshop organized by IARC in Collaboration With the US National Cancer Institute and the US Environmental Protection Agency held in Lyon, 15-17 February 1984.* T. KAKUNAGA and H. YAMASAKI (eds). *International Agency for Research on Cancer, Lyons:1985. Pp.225. Hbk ISBN 92-832-1167-7. Np.*

Trauma of the Middle Ear: Clinical Findings, Postmortem Observations and Results of Experimental Studies. By M. STROHM. *Karger:1986. Pp.254. Hbk ISBN 3-8055-4087-6. SwFr 174, DM 208, £54.40, \$74.25.*

Trends in Autonomic Pharmacology, Vol.3. STANLEY KAYSNER (ed.). *Taylor & Francis:1985. Pp.366. Hbk ISBN 0-85066-327-X. £45.*

Tropical Disease Research, Seventh Programme Report (1 January 1983-31 December 1984). *UNDP/ WORLD BANK/WHO. World Health Organization: 1985. Pp.272. Pbk ISBN 92-4-156085-1. Sfr 45.*

Archaeology

Evolutionary Case Histories from the Fossil Record. Special Papers in Palaeontology, No.33. J.C.W. COPE and P.W. SKELTON (eds). *The Palaeontological Association, London, UK:1985. Pp.202. Pbk ISBN 0038-6804. £30.*

L'Environnement au Temps de la Préhistoire. By J. RENAULT-MISKOVSKY. *Masson:1985. Pp.184. Pbk ISBN 2-225-80536-9. F 160.*

Preserving Field Records: Archival Techniques for Archaeologists and Anthropologists. By MARY-ANNE KENWORTHY, ELEANOR M. KING, MARY ELIZABETH RUWELL and TRUDY VAN HOUTEN. *The University Museum, University of Pennsylvania:1985. Pp.102. Pbk ISBN 0-934718-72-5. Np.*

Quaternary Evolution of the Great Lakes. P.F. KARROW and P.E. CALKIN (eds). *Geological Association of Canada:1985. Pp.258. Hbk ISBN 919216-29-3. \$42 (Canadian).*

General

Advances in Heat Transfer. JAMES P. HARTNETT and THOMAS F. IRVINE Jr (eds). *Academic:1985. Pp.360. Hbk ISBN 0-12-020017-1. \$79.00.*

Atti della Società Toscana di Scienza Naturali Residente in Pisa, Serie A (1984). *Grafiche Pacini Editore:1985. Pp.264. Pbk np.*

Atti della Società Toscana di Scienza Naturali Residente in Pisa, Serie B (1984). *Grafiche Pacini Editore:1985. Pbk np.*

Concert Hall Acoustics. By Y. ANDO. *Springer-Verlag:1985. Pp.151. Hbk ISBN 3-540-13505-7. DM 118.*

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Dynamical Systems: A Differential Geometric Approach to Symmetry and Reduction. By GIUSEPPE MARMO *et al.* *Wiley:1985. Pp.377. ISBN 0-471-90339-6. £36.95.*

Economic and Medicinal Plant Research, Vol. 1. H. WAGNER, HIROSHI HIKINO and NORMAN R. FARNSWORTH (eds). *Academic:1985. Pp.285. Pbk ISBN 0-12-730062-7. £60.50, \$69.50.*

Energy and Ecology. By DAVID M. GATES. *Sinauer Associates, Inc., Sunderland, Massachusetts: 1985. Pp.377. Hbk ISBN 0-87893-230-5; pbk ISBN 0-87893-231-3. Hbk £37.50; pbk £23.50.*

Explosive Remnants of War: Mitigating the Effects. ARTHUR H. WESTING (ed.). *Taylor and Francis:1985. Pp.143. Hbk ISBN 0-85066-303-2. \$25.00, £14.00.*

Frantz Fanon and the Psychology of Oppression. By HUSSEIN ABDILAH BULHAM. *Plenum:1985. Pp.299. Hbk ISBN 0-306-41950-5. Np.*

Here the People Rule: Selected Essays. By EDWARD C. BANFIELD. *Plenum:1985. Pp.348. ISBN 0-306-41969-6. Np.*

An Introduction to Twistor Theory. By S.A. HUGGETT and K.P. TOD. *Cambridge University Press:1985. Pp.145. ISBN 0-521-30879-8. Hbk £20, \$39.50; pbk £6.95, \$13.95.*

Katahdin: A Guide to Baxter State Park and Katahdin. By STEPHEN CLARK. *Thorndike Press, PO Box 157, Thorndike, Maine 04986, USA:1985. Pp.211. ISBN 0-89621-092-8. Pbk \$8.95.*

The Living House. By GEORGE ORDISH. *Bodley Head:1985. Pp.256. ISBN 0-370-30499-3. £12.95.*

Metamorphic Reactions: Kinetics, Textures and Deformation. A.B. THOMPSON and D.C. RUBIE (eds). *Springer-Verlag:1985. Pp.291. ISBN 0-387-96077-5. DM 154.*

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Plain Pictures of Plain Doctoring. By JOHN D. STOECKLE and GEORGE ABBOTT WHITE. *MIT Press:1985. Pp.250. ISBN 0-262-19236-5. \$19.95.*

Quaternary Science Reviews, Vol 3. D.Q. BOWEN (ed.). *Pergamon Press:1985. Hbk ISBN 0-08-032760-5. \$96.00, £60.00.*

Scientometric Indicators. By T. BRAUN, W. GLÄNZEL and A. SCHUBERT. *World Scientific:1985. Pp.424. ISBN 9971-966-69-7. £29.60.*

Surnames and Genetic Structure. By G.W. LASKER. *Cambridge University Press:1985. Pp.148. Hbk ISBN 0-521-30285-4. £15.00, \$24.95.*

The Toxic Time Bomb. By SAM ZIFF. *Thorsons Publishers, Wellingborough, Northamptonshire:1985. Pp.171. Pbk ISBN 0-7225-1232-5. £2.99.*

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